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=> s hansen/in
L1         2361 HANSEN/IN

=> s epirubicin
L2         2772 EPIRUBICIN

=> s l1 and l2
L3           33 L1 AND L2

=> s l3 not py>1998
          774382 PY>1998
L4             1 L3 NOT PY>1998

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L4     ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1991008770 PCTFULL . ED 20020513
TITLE (ENGLISH):         METHOD FOR ANTIBODY TARGETING OF DIAGNOSTIC OR
                         THERAPEUTIC AGENTS
TITLE (FRENCH):          PROCEDE DE CIBLAGE PAR ANTICORPS D'AGENTS DIAGNOSTIQUES
                         OU THERAPEUTIQUES
INVENTOR(S):             HANSEN, Hans, John
PATENT ASSIGNEE(S):      IMMUNOMEDICS, INC.
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER              KIND     DATE
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                         WO 9108770            A1 19910627
DESIGNATED STATES
       W:                AT AU BE CH DE DK ES FI FR GB IT JP KR LU NL NO SE
APPLICATION INFO.:       WO 1989-US5441        A  19891211


=> d kwic

L4     ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
IN     HANSEN, Hans, John

DETD   EXAMPLE 3
       Preparation of Epirubicin-glucuronide Conjugate
         Epirubicin is injected intravenously into a horse
       over a period of several weeks. Ufine is collected, and
         epirubicin glucuronide is isolated by ion-exchange
       chromatography of the urine, and purified by further
       column chromatography and/or HPLC.

       The patient is then infused intravenously with a
       sterile, pyrogen- free PBS solution containing 10 mg of
```

epirubicin glucuronide, prepared according to Example 3
hereof, on each of the next 4 days, Subsequent
- 36 -
radioimmunodetection shows significant reduction in. . .

```
=> s enzyme and l4
        107366 ENZYME
         89884 ENZYMES
        126831 ENZYME
                  (ENZYME OR ENZYMES)
L5            1 ENZYME AND L4

=> d kwic

L5      ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
IN      HANSEN, Hans, John
ABEN    The targeting capability of an antibody is enhanced using an antibody-
        enzyme conjugate and a
        separate soluble substrate-agent conjugate, wherein the targeted
        enzyme catalyzes the conversion of
        a soluble substrate, bearing at least one therapeutic or diagnostic
        agent, to a product comprising
        the. . .
ABFR    La capacite de ciblage d'un anticorps est augmentee par l'utilisation
        d'un conjugue
        anticorps-enzyme et d'un conjugue substrat soluble-agent
        separe ou l'enzyme cible catalyse la
        conversion d'un substrat soluble, comportant au moins un agent
        therapeutique ou diagnostique, en un
        produit comprenant l'agent. . .

DETD . . . DIAGNOSTIC
        OR THERAPEUTIC AGENTS
        Background Of The Invention
        The present invention relates to a method for
        enhancing the targeting capability of an antibody using an
        antibody-enzyme conjugate and a separate soluble
        substrate-agent conjugate, wherein the targeted enzyme
        catalyzes the conversion of a soluble substrate, bearing
        at least one therapeutic or diagnostic agent, to a product
        comprising the agent, which accumulates. . .

        (a) injecting a mammal parenterally with an effective
        amount for targeting and enzyme activity of an
        antibody-enzyme conjugate, the antibody being reactive
        with at least one antigen present at the-target site; and
        (b) after a sufficient period of time for the
        antibody-enzyme conjugate to localize at the target site
        and substantially clear from the circulatory system of the
        mammal, injecting the mammal parenterally with an
        effective amount for deposition at the target site of a
        soluble substrate-agent conjugate which is capable of
        transformation by the enzyme to form a product comprising
        the agent, which accumulates at the target site for
        effective treatment and/or diagnosis, the substrate-agent
        conjugate comprising a substrate for the enzyme,
        conjugated to at least one diagnostic or therapeutic
        agent,,
        wherein neither the enzyme nor an enzyme having
        similar activity with respect to the substrate-agent
        conjugate is endogenous to the mammal at a non-target site
        along the route of administration. . .
. . .
        agent on the
```

antibody and inadequate deposition of the agent at the target site. The present invention overcomes these problems by using an antibody-enzyme conjugate and a separate substrate]agent conjugate, which enables the antibody to target the site without having to load the diagnostic or therapeutic agent. . . site, according -to the present invention, can be accomplished by first injecting a mammal parenterally with an ef fective amount for targeting and enzyme activity of an antibody-enzyme conjugate and waiting a sufficient amount of time for the conjugate to localize at the target site and substantially clear from the circulatory. . . the mammal parenterally with an effective amount for deposition at the target site of a soluble substrate]agent conjugate capable of transformation by the enzyme to a product comprising the agent, which accumulates at the target site for effective treatment and diagnosis, The substrate-agent conjugate is a substrate for the enzyme, conjugated to at least one diagnostic or therapeutic agent.

The antibody component of the antibody-enzyme conjugate is the targeting portion, and serves to bind the conjugate selectively to at least one antigen present at the target site. The enzyme component of the conjugate is thereby localized at the target site. Once the non-targeted conjugate substantially clears from the bloodstream, the substrate-agent conjugate. . .

It should not encounter more than a negligible amount of the antibody-enzyme conjugate or similarly acting endogenous enzyme enroute to the target site.

However, when the substrate-agent conjugate reaches the target site, it will be transformed by the enzyme into a product comprising the diagnostic or therapeutic agent.

The enzyme can transform many molecules or subunits of substrate-agent conjugate to liberate many molecules of product in a form which will accrete at. . . favorable partition between the fluid bathing the target site and the tissue or other antigen-containing medium at the site itself. Thus, the enzyme amplifies the targeting capability of the antibody without the need to conjugate the agent to the targeting antibody,, and the agent accumulates at. . .

. . .
at a site in the body of a mammal which is of diagnostic or therapeutic interest can be used to make the antibody-enzyme conjugate for use in the method of the invention.

. . .
least one binding site specific to an antigen at a target site and at least one other binding site specific to the enzyme component of the antibody-enzyme conjugate. Such an antibody can bind the enzyme prior to injection, thereby obviating the need to covalently conjugate the enzyme to the antibody, or it can be injected and localized at the target site and, after non-targeted antibody has substantially cleared from the circulatory system of the mammal, enzyme can be injected in an amount and by a route which enables a sufficient amount of the enzyme to reach the localized antibody and bind to it to form the antibody-enzyme conjugate in situ.

. . .

immunoglobulins having more than one specificity, and by genetic engineering. The bispecific antibodies can bind to one or more epitopes on the enzyme but should not bind to a site that interferes with enzyme activity.

The enzyme used in the present invention must be capable of transforming a substantially soluble substrate-agent conjugate to form a product comprising a diagnostic and/or therapeutic agent, which accumulates at the target site. Neither the enzyme nor an enzyme with similar substrate specificity should be endogenous to the mammal along the route of administration or biodistribution of the substrate-agent conjugate.

In principle,, the enzyme can be any type of enzyme that can be bound to an antibody and can transform a substrate-agent conjugate to product, subject to the above-mentioned caviats. Proteases, glycosidases, esterases and the like are all general types of enzymes that can be used in the invention under the proper circumstances. More specific examples of suitable enzymes include, but are not limited to, glucuronidase, beta-glucosidase, beta-lactamase, cellulase, dextranase, fructase, aminopep- tidase and lysozyme.

The enzyme is selected as a function of the type of substrate-agent conjugate chosen. For example, the choice of dextran as a substrate would be coupled with the use of dextranase as the enzyme. Similarly, cellulase would be used with a cellulose substrate, A glucuronide as the
- 10 -
substrate-agent conjugate would be coupled with glucuronidase as the enzyme, and the like.

Apart from the in situ method of forming the antibody-enzyme conjaugate,, it is advantageous to covalently bind the enzyme to the antibody,, directly or through a short or long linker moiety, through one or more functional groups on the antibody and/or the enzyme, e.g., amine, carbox- yl, phenyl, thiol or hydroxyl groups.
. . .
can be used, e.g., disiocyanates, diisothiocyanates, bis(hydroxysuccinimide) esters, carbodiimides, maleimide-hydroxysuccinimide esters, glutaraldehyde and the likes
A simple, method is to mix the antibody with the enzyme in the presence of glutaraldehyde to form the antibody-enzyme conjugate. The initial Schiff base linkages can be stabilized, e.g., by borohydride reduction to secondary amines. This method is conventionally used to prepare,. . .
. . .
More selective linkage can be achieved by using a heterobifunctional linker such as a maleimide-hydroxysuccinimide ester. Reaction of the latter with an enzyme will derivatize amine groups on; the enzyme, and the
derivative can then be reacted with, e.g., an antibody Fab fragment with free sulfhydryl groups (or a larger fragment or intact immunoglobulin with sulfhydryl groups appended thereto by, e.g., Traut's Reagent),
It is advantageous to link the enzyme to a site on the antibody remote from the antigen binding site. This

can be accomplished by, e.g., linkage to cleaved interchain sulfhydryl groups, as noted above. Another method involves reacting an antibody whose carbohydrate portion has been oxidized, with an enzyme which has at least one free amine function. This results in an initial Schiff base (imine)- linkage, which is preferably stabilized by reduction. . .

Because of the size of the conjugate, it will normally be preferably to link one antibody to one enzyme molecules However, it may be advantageous to bind a plurality of antibody fragments, e.g., Fab or F(abl)2 fragments, to a single enzyme to increase its binding affinity or efficiency to the antigen target.

Alternatively, if the enzyme is not too bulky, it may be useful to link a plurality of enzyme molecules to a single antibody or antibody fragment to increase the turnover number of the conjugate and enhance the rate of deposition of. . .

Conjugates of more than one enzyme and antibody can also be used, provided they can reach the target site and they do not clear too fast. Mixtures of. . .

The antibody-enzyme conjugate can be further labeled with, or conjugated or adapted for conjugation to, a radioisotope or magnetic resonance image enhancing agent, to monitor. . .

Any conventional method of radiolabeling which is suitable for labeling proteins for in vivo use will be generally suitable for labeling antibody,s-enzyme conjugates,, and often also for labeling substrate-agent conjugates, as will be noted below. This can be achieved by direct labeling with, e.g., I-131,. . . and in Childs et al., J. Nuc. Med., 26:293 (1985)o
The substrate-agent conjugate will include a substrate, which can be transformed by the localized enzyme of the antibody-enzyme conjugate to a product. The
agent will be a diagnostic or therapeutic agent whose targeting at a specific site will be advantageous. . .
. .
13 -
lipophilic solubility will not be as important as reduction in serum solubility with cleavage of the substrate- agent conjugate by the enzyme to a product which partitions more favorably to the target. It is this partitioning out of the agent, once the substrate-agent conjugate is acted upon by the enzyme component of the targeted antibody-enzyme conjugate, so that the agent then accretes at the target site to a significantly greater extent than the substrate-agent conjugate would accrete in the absence of the enzyme, that characterizes the targeting mechanism of the invention.
. .
or
aminoacetal with the aldehyde carbon of the glucuronic
- 14 -
acid. The conjugate can be cleaved at the target site by the enzyme glucuronidase, which would be the enzyme component of the antibody-enzyme conjugate. The free drug
_.a]]

intervals along the polymer backbone. The polymer can be one that is a substrate for the enzyme component of the antibody-enzyme conjugate or it can have segments or branches that are substrates for such enzyme. The agent molecules are bound to the polymer in such a way that cleavage by the enzyme will liberate the agent, free of polymer units or bound to a small enough number of units to have the requisite lower. . .

. . .

use include, e.g.1
polyols, polysaccharides, polypeptides and the like. one type of polysaccharide is dextran, an alpha-glycoside, which can be cleaved with the enzyme dextranase. The diagnostic or therapeutic agent can be functionalized to contain reactive groups towards the dextran hydroxyls,, e.g., anhydrides, isocyanates or isothiocyanates and. . .

. . .

and
subsequent amine groups, is less advantageous because the
- 16 -
polymer then behaves more like poylysine and may also be resistant to enzyme cleavage. A lower number results in less than desirable loading of drug,, toxin,, chelator or boron addend, which may be disadvantageous, especially if the turnover number of the enzyme is low,
The oxidized dextran is then -reacted with a polyamine, preferably a diamine, and more preferably a mono- or poly-hydroxy diamine. Suitable. . .

. . .

cells, tissues
or other structures, e.g., atherosclerotic plaque, fibrin clot, virus particle, parasite and the like), and upon the ef f iciency of enzyme cleavage of substrate molecules or subunits to liberate a product comprising the drug which has a sufficiently favorable partition coefficient to the target. . . of
from about 3 to about 25, although these are preferred and not limiting amounts. Very heavy loading of drug molecules can inhibit enzyme activity due primarily to steric hindrance to binding of the substrate conjugate to the active site of the enzyme. Too light loading can result in insufficient reduction in fluid solubility for the drug as a result of enzyme cleavage since a smaller portion of the polysaccharide-drug conjugate might diffuse away from the bound enzyme before enough sugar subunits are cleaved off to reduce solubility enough for the drug (with perhaps a few glycoside subunits still bound. . .

. . .

a therapeutic
dosage of alpha particles to the targeted tissue upon thermal neutron irradiation, even when the percentage of an injected dose of antibody-enzyme conjugate which localizes in the target tissue is relatively low,, e.g., 1-10%. Such localization percentages are not uncommon for antibody-targeted species.

. . .

The substrate-agent conjugate may incorporate
functions whose primary purpose is to improve the lipid solubility and decrease the water solubility of the resultant enzyme cleavage product containing the boron addend,
It is useful to employ boron cage compounds to make such conjugates, because of their relative ease. . .

. . .

to an alpha-glycoside such as dextran

or aminodextran is a beta-glycoside such as carboxymethyl-cellulose (CMC), which can be cleaved by a cellulase enzyme. Attachment of diagnostic or therapeutic agents to the CMC will be analogous to the method used for dextran, since both are sugar. . .

Yet another variant on the polymer substrate is a polymer that is not cleaved by the enzyme, but which bears short linker segments of an oligomer that is a substrate
- 23 -
for the enzyme, and which bears drugs,, chelators, boron addends and like diagnostic or therapeutic agents, As one illustration, a polyvinyl alcohol could be used. . .
. . .
residues on the carrier. A
short polyester or oligopeptide linker can be used instead of an oligosaccharide linker, with an esterase or peptidase enzyme that cleaves the linker. The ordinary skilled artisan will be able to envision other variants
-
that fall within the broad scope of. . . for the target, in unmodified form, but which is then modified by conjugation to solubilizing substrate molecules which are then cleaved by targeted enzyme. one illustration of this subgeneric type of substrate-agent conjugate is a polylysine to which are bound a plurality of radiometal or paramagnetic. . . and becomes less soluble in the fluid
bathing the target site after the coating molecules are cleaved by action of the localized enzyme of the antibody-enzyme conjugate.

It will be appreciated that clearance of the antibody-enzyme conjugate and/or the substrate-enzyme conjugate can be accelerated, after a sufficient time for localization or deposit of the diagnostic or therapeutic agent, by using a second antibody. . . antibody clearance can be determined with
the aid of a label on either conjugate, so that the extent of localization of the antibody-enzyme conjugate at the target site and/or the extent of deposit of the agent at the target site, and the biodistribution of non-targeted. . .
. . .
therapeutic and
diagnostic use. The first injectable preparation contains an effective amount of an antibody or antibody fragment
- 26 -
conjugated to an enzyme, in a pharmaceutically acceptible injection vehicle, preferably phosphate-buffered saline (PBS) at physiological pH and concentrationt The second injectable preparation contains an effective amount. . .
. . .
using two suitable containers. The f irst
container has an effective amount of an antibody or antibody fragment covalently bound to an enzyme. The second container has an ef fective amount of a soluble substrate conjugated to at least one therapeutic or diagnostic agent. The reagents. . .

The above illustrates the general methods of administration of antibody-enzyme conjugates and substrate-therapeutic or diagnostic agent conjugates according to the present invention. It will be appreciated that the modes of administration of the. . . may not be the same, since the

clearance pathways and biodistributions of the conjugates will generally differ. For example, intraperitoneal administration of an antibody-enzyme conjugate may be advantageous for targeting an ovarian tumor, whereas intravenous administration of a radioisotope-substrate conjugate for imaging may be desirable because of. . .

The antibody-enzyme conjugate will generally be administered as an aqueous solution in PBS, preferably a sterile solution, especially if it is for use in. . .

Advantageously, dosage units of about 50 micrograms to about 5 mg of the antibody-enzyme conjugate will be administered, either in a single dose or in divided doses, although smaller or larger doses may be indicated in particular. . .

. . .

time frames to localize at the target site, and the above time frames may be affected by the presence of the conjugated enzyme.

Again, it is noted that labeling the antibody-enzyme conjugate permits monitoring of localization and clearance.

Normally,, it will be necessary for at least about 0.0001% of the injected dose of antibody-enzyme conjugate to localize at the target site prior to administration of the substrate-agent conjugate. To the extent that a higher targeting efficiency for. . .

It follows that an effective amount of an antibody-enzyme conjugate is that amount sufficient to target the conjugate to the antigen at the target site and 35- thereby bind an amount of the enzyme sufficient to transform enough of the soluble substrate-agent conjugate to product to result in accretion of an effective

- 29 -

diagnostic or therapeutic. . . be a sterile solution if intended for human use. The substrate-agent conjugate will be administered after a sufficient time has passed for the antibody]enzyme conjugate to localized at the target site and substantially clear from the circulatory system of the mammal.

. . .

effective amount of the agent to the target site and that amount of a substrate which will be capable of transformation by the enzyme to a form of the product that tends to accumulate at the target site. An effective amount of a therapeutic or diagnostic. . .

. . .

I-131, I-123, Tc-99m, In-111 and Ga The antibody will be one that binds to an antigen at the target site, and the enzyme will be one that converts the substrate-agent conjugate to a product that accretes at the target site in an amount sufficient to permit. . .

. . .

site for high contrast MRI enhancement, the polylysine will be loaded with a higher amount of chelators and more of the

- 32 -

antibody-enzyme conjugate and the substrate-chelate conjugate will be administered in order to deposit a high

concentration of paramagnetic ions atthe target site.

Use of a labeled antibody-enzyme conjugate to estimate the amount of enzyme localized at the target site will also aid in dosimetric analysis.

. . .
studies,, to optimize the dose of substrate-agent conjugate, as a function of accessibility of the site, mode of administration,, turnover number of the enzyme,, desired dose of the agent to the site,, and rate of clearance of non-targeted conjugate. This will be expected and the techniques. . .

EXAMPLE 3
Preparation of Epirubicin-glucuronide Conjugate
Epirubicin is injected intravenously into a horse over a period of several weeks. Ufine is collected, and epirubicin glucuronide is isolated by ion-exchange chromatography of the urine, and purified by further column chromatography and/or HPLC.

EXAMPLE 4
Preparation of Antibody-enzyme Conjugate
(A) A substantially monoconjugated enzyme-antibody preparation is prepared by mildly oxidizing the carbohydrate portion of an anti-CEA 1gG with periodate, then contacting the oxidized IgG with a dilute solution of dextranase (from Penicillium sp., Worthington Biochemical Corp.,, Freehold,, -NJ;) to produce an antibody-enzyme conjugate, which is then stabilized by
- 35 -
borohydride, in the usual manner. The conjugate can be radiolabeled with I-131, by conventional. . .

The patient is then infused intravenously with a sterile, pyrogen- free PBS solution containing 10 mg of epirubicin glucuronide, prepared according to Example 3 hereof, on each of the next 4 days, Subsequent
- 36 -
radioimmunodetection shows significant reduction in. . .

CLMEN. . . or
therapeutic agent to a target site, which comprises the steps of:
(a) injecting a mammal parenterally with an effective amount for targeting and enzyme activity of an antibody-enzyme conjugate, said antibody being reactive with at least one antigen present at the target site; and
(b) after a sufficient period of time for said antibody-enzyme conjugate to localize at the target site and substantially clear from the circulatory system of the mammal, injecting said mammal parenterally with an effective amount for deposition at said site of a soluble substrate-agent conjugate which is capable of transformation by said enzyme to form a product comprising at least one diagnostic or therapeutic agent, which accumulates at said target site for effective treatment or diagnosis, said substrate-agent conjugate comprising a substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent,
wherein neither said enzyme nor an enzyme having similar activity with respect to said substrate-agent conjugate is endogenous to said mammal at a non-target site along the route of administration. . . in an amount which interferes with targeting and accretion of said agent,

2a The method of claim 1, wherein the antibody in said antibody-enzyme conjugate specifically binds to an antigen produced by or associated with a tumor, an infectious or parasitic lesion, a fibrin clot, a myocardial. . .

3 The method of claim 1, wherein the enzyme in said antibody-enzyme conjugate is a protease, a glycosidase, a glucuronidase or an esterase.
- 39 -
The method of claim 3, wherein said enzyme is a dextranase, a cellulase or a glucuronidase.
. . .
claim 13, wherein said substrate is a dextran, an aminodextran, a carboxymethylcellulose or a polypeptide.
15o The method of claim 13, wherein said enzyme is a dextranase or a cellulase, and wherein said substrate-agent conjugate comprises a non-substrate aminodextran or a polylysine carrier, to which is conjugated said. . . of said agent,, and which is further conjugate to at least one solubilizing dextran or carboxymethyleellulose oligomer which is a substrate for said enzyme.
16o The method of claim 13, wherein said substrate-agent conjugate comprises a non-substrate polymer to which is attached at least one substrate oligomer to. . .

17 The method of claim 1, wherein said antibody-enzyme conjugate is further conjugated to or adapted for conjugation to a radioisotope or magnetic resonance image enhancing agent, to -monitor the clearance of antibody-enzyme conjugate from the circulatory system or its localization at the target site.
18* The method of claim 1, wherein said substrate-agent conjugate. . .
. . .
a therapeutic or diagnostic agent to a target site, comprising;
(a) a first sterile injectable solution containing an effective amount for targeting and enzyme activity of an antibody-enzyme conjugate,, said antibody being reactive with at least one antigen present at the target site, in a pharmaceutically acceptible sterile injection vehicle;. . .
injectable solution containing an effective amount for deposition at said site of a soluble substrate-agent conjugate which is capable of transformation by said enzyme to form a product comprising at least one diagnostic or therapeutic agent, said substrate]agent conjugate comprising a substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent,, wherein neither said enzyme nor an enzyme having similar activity with respect to said substrate-agent conjugate is endogenous to the mammal at a non]target site along the route of. . . or diagnostic agent to a target site, comprising, in suitable containers;
(a) a first sterile container containing an effective amount for targeting and enzyme activity of an antibody-enzyme conjugate, said antibody being reactive with at least one antigen present at the target site; and
(b) a second sterile container containing an effective amount for deposition at said site of a soluble substrate-agent conjugate which is capable of transformation by said enzyme to form a product comprising

at least one diagnostic or therapeutic agent, said
- 42 -
substrate-agent conjugate comprising a substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent, wherein neither said enzyme nor an enzyme having similar activity with respect to said substrate-agent conjugate is endogenous to the mammal at a non-target site along the route of. . .

23 The kit of claim 21, wherein said antibody-enzyme conjugate is further conjugated to,, or adapted for conjugation to a radioisotope or magnetic resonance image enhancing agent.

. .
or adapted for
conjugation to a radioisotope, magnetic resonance image enhancing agent or other label.
25e A method according to Claim 11 wherein said antibody-enzyme conjugate provided in step (a) comprises a bispecific antibody or antibody fragment having a first binding site specific to said antigen present at a target site and a second binding site specific to an epitope on said enzyme which does not interfere with enzyme activity,
said bispecific antibody or antibody fragment being non-covalently bound to said enzyme at said second binding site to form said antibody-enzyme conjugate.
- 43

26 A sterile injectable preparation for human use, for targeting a therapeutic or diagnostic agent to a target site, comprising;
(a) a first sterile injectable solution containing an effective amount, for targeting and enzyme activity, of the antibody-enzyme conjugate of Claim 25, dissolved in a pharmaceutically acceptable sterile injection vehicle; and
(b) a second sterile injectable solution containing an effective amount, for deposition at said site, of a soluble substrate-agent conjugate which is capable of transformation by said enzyme to form a product comprising at least one diagnostic or therapeutic agent, said substrate-agent conjugate comprising a substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent, wherein neither said enzyme nor an enzyme having similar activity with respect to said substrate-agent conjugate is endogenous to a human at a non-target site along the route of administration. . . targeting a therapeutic or diagnostic agent to a
target site, comprising;
(a) a first sterile container containing an effective amount, for targeting and enzyme activity, of the antibody-enzyme conjugate of Claim 25; and
(b) a second sterile container containing an effective amount, for deposition at said site, of a soluble substrate-agent conjugate which is capable of transformation by said enzyme to form a product comprising at least one diagnostic or therapeutic
- 44 -
agent,, said substrate-agent conjugate comprising a-substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent, wherein neither said enzyme nor an enzyme having similar activity with respect to said substrate-agent

conjugate is endogenous to a human at a non-target site along the route of administration. . .

. . .

having a first binding site specific for an antigen present at a target site and a second binding site specific for an enzyme;
(b) injecting a mammal parenterally with an effective amount for targeting of said antibody or antibody fragment;
(c) after a sufficient period of time. . . localize at the target site and substantially clear from the circulatory system of the mammal, injecting said mammal parenterally with an effective amount for enzyme activity of said enzyme,, such that said localized antibody binds said enzyme to form said antibody-enzyme conjugate in situt and
(d) further injecting said mammal parenterally with an effective amount for deposition at said site
-
of a soluble substrate agent conjugate which is capable of transf ormation by said enzyme to form a product comprising at least one diagnostic or therapeutic agent, which accumulates at said target site for effective treatment or diagnosis, said substrate-agent conjugate comprising a substrate for said enzyme, conjugated to said at least one diagnostic or therapeutic agent;
wherein neither said enzyme nor an enzyme having similar
activity with respect to said substrate-agent conjugate is endogenous to said mammal at a non-target site along the route of administration. . .

32 The method of Claim 31, wherein said antibody or antibody fragment in said antibody-enzyme conjugate specifically binds to an antigen produced by or associated with a tumor, an infectious or parasitic lesion, a fibrin clot, a myocardial infarction, an atherosclerotic plaque, a non-cancerous cell or a damaged normal cell.
3 3 eThe method of Claim 31, wherein the enzyme in said antibody-enzyme conjugate is a protease, a glycosidase, a glucuronidase or an esterase,

35 The method of claim 31, wherein said enzyme is a dextranase or a cellulose, and wherein said substrate-agent conjugate comprises a non-substrate aminodextran or a polylysine carrier, to which is. . . said agent, and which is further
- 46
conjugated to at least one solubilizing dextran or carboxymethylcellulose oligomer which is a substrate for said enzyme.

. . .

fragment having a
first binding site specific for an antigen present at a target site and a second binding site specific for an enzyme, said antibody or antibody fragment being dissolved in a pharmaceutically acceptable sterile injection vehicle;
(b) a second sterile injectable solution containing an effective amount for enzyme activity at said target site of said enzyme, said enzyme being dissolved in a pharmaceutically acceptable sterile injection vehicle; and
(c) a third sterile injectable solution

containing an effective amount, for deposition at
said site, of a soluble substrate-agent conjugate
which is capable of transformation by said enzyme to
form a product comprising at least one diagnostic or
therapeutic agent, said substrate-agent conjugate
comprising a substrate for said enzyme, conjugated to
said at least one diagnostic or therapeutic agent,,
said substrate-enzyme conjugate being dissolved in a
pharmaceutically acceptable sterile injection
vehicle;
wherein neither said enzyme nor an enzyme having
similar
activity with respect to said substrate-agent conjugate is
endogenous to a human at a non-target site along the route
- 47. . .
. . .
having a first binding
site specific for an antigen present at a target site
and a second binding site specific for an enzyme;
(b) a second sterile container containing an
effective amount for enzyme activity at said target
site of said enzyme; and
(c) a third sterile container containing an
effective amount for deposition at said site of a
soluble substrate]agent conjugate which is capable of
transformation by said enzyme to form a product
comprising at least one diagnostic or therapeutic
agent, said substrate-agent conjugate comprising a
substrate for said enzyme, conjugated to said at
least one diagnostic or therapeutic agent;
wherein neither said enzyme nor an enzyme having
similar
activity with respect to said substrate]agent conjugate is
endogenous to a human at a non-target site along the route
of administration. . .

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=> s l4 and bispecific
          5901 BISPECIFIC
            50 BISPECIFICS
          5901 BISPECIFIC
                 (BISPECIFIC OR BISPECIFICS)
L6           1 L4 AND BISPECIFIC

=> d kwic bispecific
'BISPECIFIC' IS NOT A VALID FORMAT FOR FILE 'PCTFULL'

The following are valid formats:

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ENTER DISPLAY FORMAT (STD):end

=> d kwic

L6 ANSWER 1 OF 1 PCTFULL COPYRIGHT 2006 Univentio on STN
IN HANSEN, Hans, John

DETD . . . fusions of more than one clone to form polyomas that produce immunoglobulins having more than one specificity, and by genetic engineering. The bispecific antibodies can bind to one or more epitopes on the enzyme but should not bind to a site that interferes with enzyme activity.

EXAMPLE 3
Preparation of Epirubicin-glucuronide Conjugate
Epirubicin is injected intravenously into a horse over a period of several weeks. Ufine is collected, and epirubicin glucuronide is isolated by ion-exchange chromatography of the urine, and purified by further column chromatography and/or HPLC.

The patient is then infused intravenously with a sterile, pyrogen- free PBS solution containing 10 mg of epirubicin glucuronide, prepared according to Example 3 hereof, on each of the next 4 days, Subsequent
- 36 -
radioimmunodetection shows significant reduction in. . .

CLMEN. . . resonance image enhancing agent or other label.
25e A method according to Claim 11 wherein said antibody-enzyme conjugate provided in step (a) comprises a bispecific antibody or antibody fragment having a first binding site specific to said antigen present at a target site and a second binding site specific to an epitope on said enzyme which does not interfere with enzyme activity, said bispecific antibody or antibody fragment being non-covalently bound to said enzyme at said second binding site to form said antibody-enzyme conjugate.
- 43
. . .
cytokine, radiosensitizer or photosensitizer.
29a A method for targeting a diagnostic or therapeutic agent to a target site, comprising the steps of**
(a) providing a bispecific antibody or antibody fragment having a first binding site specific for an antigen present at a target site and a second binding site. . .
. . .
a therapeutic or diagnostic agent to a target site, comprising;
(a) a first sterile injectable solution containing an effective amount for targeting of a bispecific antibody or antibody fragment having a first binding site specific for an antigen present at

```
      a target site and a second binding.  .  .
 .  .  .
      a therapeutic or diagnostic agent to a
      target site, comprising;
      (a) a first sterile container containing an
      effective amount for targeting of a bispecific
      antibody or antibody fragment having a first binding
      site specific for an antigen present at a target site
      and a second binding site.  .  .


=> s l4 and hapten
          6571 HAPTEN
          5066 HAPTENS
          9370 HAPTEN
                 (HAPTEN OR HAPTENS)
L7           0 L4 AND HAPTEN

=>

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COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
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 NEWS  7  MAY 19  Derwent World Patents Index to be reloaded and enhanced
 NEWS  8  MAY 30  IPC 8 Rolled-up Core codes added to CA/CAplus and
                  USPATFULL/USPAT2
 NEWS  9  MAY 30  The F-Term thesaurus is now available in CA/CAplus
 NEWS 10  JUN 02  The first reclassification of IPC codes now complete in
                  INPADOC
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 NEWS 11  JUN 26  TULSA/TULSA2 reloaded and enhanced with new search and
                  and display fields
 NEWS 12  JUN 28  Price changes in full-text patent databases EPFULL and PCTFULL
 NEWS 13  JUl 11  CHEMSAFE reloaded and enhanced
 NEWS 14  JUl 14  FSTA enhanced with Japanese patents
 NEWS 15  JUl 19  Coverage of Research Disclosure reinstated in DWPI

 NEWS EXPRESS  JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 26 JUNE 2006.

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=> file pctfull
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
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FULL ESTIMATED COST                                         0.21       0.21

FILE 'PCTFULL' ENTERED AT 11:06:52 ON 28 JUL 2006
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FILE LAST UPDATED:          25 JUL 2006      <20060725/UP>
MOST RECENT UPDATE WEEK:        200629        <200629/EW>
FILE COVERS 1978 TO DATE

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    SEE
    http://www.stn-international.de/stndatabases/details/ipc-reform.html >>>

>>> FOR CHANGES IN PCTFULL PLEASE SEE HELP CHANGE
    (last updated April 10, 2006) <<<

>>> NEW PRICES IN PCTFULL AS OF 01 JULY 2006. FOR DETAILS,
    PLEASE SEE HELP COST <<<

=> s camptothecin
          3437 CAMPTOTHECIN
           531 CAMPTOTHECINS
L1        3617 CAMPTOTHECIN
                 (CAMPTOTHECIN OR CAMPTOTHECINS)

=> s l1 and prodrug
         14822 PRODRUG
         14920 PRODRUGS
         19302 PRODRUG
                 (PRODRUG OR PRODRUGS)
L2        1569 L1 AND PRODRUG
```

```
=> s antibod?
L3       89692 ANTIBOD?

=> s l3 and l2
L4        1316 L3 AND L2

=> s enzyme
        107366 ENZYME
         89884 ENZYMES
L5      126831 ENZYME
                 (ENZYME OR ENZYMES)

=> s esterase
          5528 ESTERASE
          3978 ESTERASES
L6        8323 ESTERASE
                 (ESTERASE OR ESTERASES)

=> s l6 and l4
L7         130 L6 AND L4

=> s adept and l2
          1088 ADEPT
             9 ADEPTS
          1097 ADEPT
                 (ADEPT OR ADEPTS)
L8          74 ADEPT AND L2

=> s l8 not py>1998
        774382 PY>1998
L9           1 L8 NOT PY>1998

=> d kwic

L9     ANSWER 1 OF 1     PCTFULL   COPYRIGHT 2006 Univentio on STN
TIEN   PHARMACEUTICAL COMPOSITIONS CONTAINING ANTIBODY-ENZYME CONJUGATES IN
       COMBINATION WITH PRODRUGS
ABEN   Prodrugs which can be activated by enzymes, are formulated for
       sequential administration, with
       enzyme conjugates. Either or each component comprises a polymeric
       carrier which allows it to be
       directed preferentially to the target tissue. A new polymer-
       prodrug conjugate is cleavable by
       Cathepsin B or other thiol-dependent protease. The invention is of
       particular utility for targeting
       solid tumours.

DETD   PHARMACEUTICAL COMPOSITIONS CONTAINING ANTIBODY-ENZYME CONJUGATES IN
       COMBINATION WITH
         PRODRUGS
       Field of the Invention
       The present invention relates to compositions and kits
       for use in enzyme-prodrug therapy, in which the enzyme is
       conjugated to a carrier.

         Prodrugs have been used for many years in medicine to
       treat a variety of disorders, They can offer improved
       solubility, improved pharmacokinetics and tissue
       distribution, avoidance of unfavourable metabolism,
       selective organ effects and specific tumour toxicity. In
       order to use prodrugs as anticancer agents, the tumour must
       have a high level of the enzyme that activates the prodrug,
       or a foreign enzyme must be delivered, and no activating
       enzyme must be present in normal tissues. The prodrug must
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be innocuous and pharmacodynamically inert and be a substrate for the enzyme with favourable Km and Vmax values.

The concepts of Antibody Directed Enzyme Prodrug Therapy (ADEPT) (refs. 1,2) and Gene/Viral Directed Enzyme Prodrug Therapy (G/VDEPT) (ref. 3) using either antibody conjugates or a retroviral vector to deliver an enzyme to a tumour,, are already well established, In ADEPT , a foreign enzyme which metabolises substrates not normally metabolised by mammalian cells is linked chemically to a tumour-specif ic or tumour-associated antibody;. . .
. . .
control of a tumour-specif ic promoter. The f oreign enzyme is then used to activate a carefully designed low molecular weight prodrug, Animal models and pilot human studies have proven that it is possible to deliver selectively to a solid tumour an activating enzyme such. . .

In the case of ADEPT, these include the immunogenicity of the antibody-enzyme conjugate,, the need to tailor each conjugate to target antigen present on the tumour,, the difficulty in optimisation of the dosing schedule, and the need to use a clearing antibody in the case of ADEPT (ref.
. . .
the difficulty of evaluating the duration and reproducibility of enzyme expression on a patient basis, leading to difficulties in optimising the schedule of prodrug follow-up.

The second component is a prodrug, e.g. a composition that comprises a pharmaceutically-acceptable excipient and a prodrug, The prodrug is typically substantially inactive
(in terms of drug activity) but capable of being activated by the enzyme,
It is to be noted that. . .

The two components of the kit are used in a treatment regimen similar to ADEPT, except that the enzyme conjugate is administered in place of the antibody-enzyme conjugate.
. . .
one significant difference is that the two components can be administered, in use of the present invention, in either order. For instance, prodrug-containing composition may be given first. The present invention can avoid the problems with ADEPT, resulting from the immunogenicity of the antibody-enzyme conjugate. Furthermore, although a polymer carrier is not specifically targeted to antigenic sites of the tumour cell surface, it is nevertheless expected to preferentially accumulate in solid tumours through the EPR effect. It has been observed that ADEPT is surprisingly non-specific in vivo, and it is believed that the polymer-enzyme conjugate is likely to exhibit preferential accumulation as significant as that exhibited in ADEPT.

Description of the Drawings
Figure 1 shows the reaction mechanism of drug release from cephalosporin-linked prodrug by 0-lactamase;
Figure 2 shows the reactions of Examples 1 and 2;

Figure 3 is a graph showing spectra f. . . chart showing tumour accumulation of

free and conjugated radiolabelled fl-lactamase in C57 mice bearing B16FIO melanoma;
Figure 7 shows the structure of the prodrug PK1 cleavable by cathepsin B;
Figure 8 is a graph showing the release of doxorubicin from HPMA-Gly-Phe-Leu-Gly-dox by free and conjugated cathepsin-B and without;
Figure. . .

. . .

interest. Whilst the use of such an antibody-polymer-enzyme conjugate may have some advantages over the antibody-enzyme conjugate used in the normal ADEPT system in terms of reduced immunogenicity, the present invention preferably avoids the use of active targeting ligands for the enzyme conjugate.

. . .

significant level of enzyme activity. The level may be reduced as compared to the native enzyme, provided that significant useful activity is retained,
The prodrug used in the invention may be a relatively low molecular weight compound. The action of the enzyme on the prodrug may not significantly change the molecular weight of the compound. A--useful summary of suitable prodrugs and the enzymes which activate the prodrugs is
given in Table 1 of reference 5, a review article citing many references in which full details of the prodrugs and their use are described. Thus the enzyme may be selected from DT diaphorase,, plasmin,, carboxypeptidase G2,, thymidine kinase (viral), cytosine deaminase, glucose. . . Many cytotoxic agents can
be attached, provided that they have NH2 or OH groups that can be substituted and thus act as prodrugs, The rate of the hydrolysis is measured in vitro by the change in absorbance.

The prodrug should be substantially inactive in terms drug activity. Thus the activated drug should preferably have an activity which is at least 100 times that of the prodrug, most preferably at least 1000 times the activity.

. . .

treatment of cancers, Thus the drug may be any known cytotoxic agent capable of being provided in the f orm of a prodrug for enzymic activation.

A suitable list of drugs and their prospective prodrugs, as well as the respective activating enzymes is given in reference 5, Table 1, Thus the drug may be selected from.

. . .

Doxorubicin and
Daunorubicin
4-Desacetylvinblastine carboxhydrazide
Nitrogen mustards (various)
Mitomycin alcohol
Etoposide
Palytoxin
Melphalan
5-(Aziridin yl) hydroxylamino nitrobenzamide
Actinomycin D, mitomycin C
Taxanes, such as taxol and taxotere
Topoisomerase inhibitors such as camptothecin and topotecan.

Pref erably the prodrug comprises a carrier to which the drug is conjugated. In some circumstances, it may be desirable f or the enzymic activation of the prodrug not to be accompanied by release of activated drug from the polymer conjugate. Thus an activated drug-polymer conjugate may have polymer bound to. . . through linker moieties which are not cleaved by the enzyme, which allow the drug's activity to be retained. Alternatively, the activation of the prodrug through reaction of the enzyme may take place independently of cleavage of the drug moiety from the polymer conjugate, for instance through activity. . .

It is , however , pref erred f or activation of a prodrug-polymer conjugate to occur by cleavage of a linker which is susceptible to cleavage by the enzyme.

In another embodiment of the invention,, a prodrug comprises a conjugate of a polymer and a drug moiety joined together by a peptidyl linker, and an enzyme conjugate comprises an enzyme bound to a carrier moiety. The conjugate and the prodrug are components of a novel kit comprising two compositions, arranged for sequential administration, each containing one of the aforementioned conjugates.

. . .
conjugate as used in the first aspect of the invention. It may also be an antibody-enzyme conjugate, for instance as used in ADEPT. Alternatively, the carrier may be another type of molecule capable of being actively targeted to desired target cells, or to which other proteins. . .

. . .
The enzyme used in this aspect of the invention is a peptidase enzyme capable of cleavage of the peptidyl linker of the prodrug conjugate. Cleavage of the linker preferably results in release of active drug.

A further aspect of the present invention lies in the use of thiol-dependent proteases as the enzyme component of enzyme/prodrug therapy. A novel conjugate consists of a thiol-dependent protease covalently bound to a carrier, said conjugate retaining thiol-dependent protease activity.

A product or kit comprises the novel conjugate and a prodrug comprising a drug bound to a carrier via a linker cleavable by the thiol-dependent protease,
In each aspect of the invention, the. . .

. . .
period of less than, for instance, 12 hours, preferably less than 6 hours (the circulation half-life), It is particularly convenient for the prodrug conjugate to be administered first, to achieve rapid accumulation in the tumour and subsequent clearance from the circulation of the potentially toxic prodrug by excretion through the kidneys, This is a significant improvement over the ADEPT system where the enzyme has to administered first.

Preferably the first component to be administered in the novel method is the prodrug. It should therefore be adapted to optimise its EPR and hence tumour accumulation.

. . .
EPR

effect, The circulation time of the enzyme conjugate should be sufficiently long for activation at the desired site of substantially all the prodrug. The difference in time between the administration of the two compositions may be hours or even days. Controlling the molecular weight of the. . .

An advantage of the third aspect of the present invention is that the prodrug, when preferentially accumulated in the tumour, will be activated intra-cellularly by endogenous thiol-dependent protease, present in increased levels inside the cells in. . .

. . .
Vmax is 0,20 M/s/unit and
Km is 0.07,
. The conjugate of Example 1 is suitable for use in combination with a suitable prodrug.

The cathepsin-B conjugate of Example 2 is suitable for use in combination with a suitable prodrug such as HPMA-Gly-Phe-Leu-Gly-doxorubicin (PKI; see Fig. 7)o The activity of cathepsin-B (free or conjugated) on the high molecular weight substrate PK1 was assessed. . .

CLMEN I I A product comprising, as a combined preparation for sequential administration, in drug treatment, an enzyme conjugate and a prodrug, the enzyme conjugate consisting of a functional enzyme covalently bound to a polymeric carrier, and the prodrug being substantially inactive (in terms of drug activity) but capable of being activated by the enzyme.

2 A product according to claim 1, in which the prodrug comprises a carrier to which the drug is covalently conjugated.
3* A product according to claim 2, in which the carrier of the prodrug is a polymer.

4 A product according to claim 3, in which the enzyme is a protease and the drug is conjugated to the. . . cleavable by a protease enzyme.
5* A product comprising, as a combined preparation for sequential administration, in drug treatment, an enzyme conjugate and a prodrug, the prodrug comprising a drug
bound to a polymeric carrier by a peptide linker which is cleavable by the enzyme.
6* A product according to. . .

. . .
which
the enzyme is 0-lactamase.
96 A product comprising, as a combined preparation for sequential administration in drug treatment, an enzyme conjugate and a prodrug, the enzyme conjugate consisting of functional thiol-dependent protease covalently bound to carrier, and the prodrug comprising a drug bound to a carrier by a peptide linker which is a substrate for the thiol-dependent protease,

18 Use of a prodrug as defined in any of claims 1 to 5, 9 and 12, for the manufacture of a medicament for use in therapy associated with the active drug, the therapy comprising the separate administration of an enzyme that releases the drug from the prodrug.
19 . Use according to claim 18, in which the prodrug is

administered before the enzyme conjugate.

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L9     ANSWER 1 OF 1      PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:         1998056425 PCTFULL  ED 20020514
TITLE (ENGLISH):          PHARMACEUTICAL COMPOSITIONS CONTAINING ANTIBODY-ENZYME
                          CONJUGATES IN COMBINATION WITH PRODRUGS
TITLE (FRENCH):           COMPOSITIONS PHARMACEUTIQUES CONTENANT DES CONJUGUES
                          ANTICORPS-ENZYMES COMBINES A DES PROMEDICAMENTS
INVENTOR(S):              DUNCAN, Ruth;
                          SATCHI, Ronit
PATENT ASSIGNEE(S):       THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON;
                          DUNCAN, Ruth;
                          SATCHI, Ronit
LANGUAGE OF PUBL.:        English
DOCUMENT TYPE:            Patent
PATENT INFORMATION:
                          NUMBER              KIND     DATE
                          ---------------------------------
                          WO 9856425            A1 19981217
DESIGNATED STATES
       W:                 AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE
                          ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC
                          LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU
                          SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH
                          GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT
                          BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF
                          BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.:        WO 1998-GB1700        A  19980611
PRIORITY INFO.:           GB 1997-97304070.2       19970611


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     FILE 'PCTFULL' ENTERED AT 11:06:52 ON 28 JUL 2006
L1          3617 S CAMPTOTHECIN
L2          1569 S L1 AND PRODRUG
L3         89692 S ANTIBOD?
L4          1316 S L3 AND L2
L5        126831 S ENZYME
L6          8323 S ESTERASE
L7           130 S L6 AND L4
L8            74 S ADEPT AND L2
L9             1 S L8 NOT PY>1998

=> s l6 and target?
        180866 TARGET?
L10       5283 L6 AND TARGET?

=> s l10 and l7
L11         128 L10 AND L7

=> s l11 not py>1998
        774382 PY>1998
L12           3 L11 NOT PY>1998

=> d ibib 1-3

L12    ANSWER 1 OF 3      PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:         1998056425 PCTFULL  ED 20020514
TITLE (ENGLISH):          PHARMACEUTICAL COMPOSITIONS CONTAINING ANTIBODY
```

-ENZYME CONJUGATES IN COMBINATION WITH PRODRUGS

TITLE (FRENCH): COMPOSITIONS PHARMACEUTIQUES CONTENANT DES CONJUGUES ANTICORPS-ENZYMES COMBINES A DES PROMEDICAMENTS

INVENTOR(S): DUNCAN, Ruth;
SATCHI, Ronit

PATENT ASSIGNEE(S): THE SCHOOL OF PHARMACY, UNIVERSITY OF LONDON;
DUNCAN, Ruth;
SATCHI, Ronit

LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9856425	A1	19981217

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-GB1700 A 19980611
PRIORITY INFO.: GB 1997-97304070.2 19970611

L12 ANSWER 2 OF 3 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1998035554 PCTFULL ED 20020514

TITLE (ENGLISH): COMBINED TUMOR SUPPRESSOR GENE THERAPY AND CHEMOTHERAPY IN THE TREATMENT OF NEOPLASMS

TITLE (FRENCH): COMBINAISON THERAPIE GENIQUE SUPPRESSIVE DE TUMEURS - CHIMIOTHERAPIE UTILISEE DANS LE TRAITEMENT DE NEOPLASMES

INVENTOR(S): NIELSEN, Loretta;
HOROWITZ, Jo, Ann;
MANEVAL, Daniel, C.;
DEMERS, G., William;
RYBAK, Mary, Ellen;
RESNICK, Gene

PATENT ASSIGNEE(S): CANJI, INC.;
NIELSEN, Loretta;
HOROWITZ, Jo, Ann;
MANEVAL, Daniel, C.;
DEMERS, G., William;
RYBAK, Mary, Ellen;
RESNICK, Gene

LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9835554	A2	19980820

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-US3514 A 19980217
PRIORITY INFO.: US 1997-8/801,285 19970218
US 1997-8/801,681 19970218
US 1997-8/801,755 19970218
US 1997-8/801,765 19970218
US 1997-60/038,065 19970218

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                        US 1997-60/047,834      19970528

L12    ANSWER 3 OF 3     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1996001127 PCTFULL  ED 20020514
TITLE (ENGLISH):         CAMPTOTHECIN DRUG COMBINATIONS AND
                         MEDICAMENTS WITH REDUCED SIDE EFFECTS
TITLE (FRENCH):          COMBINAISONS MEDICAMENTEUSE CONTENANT DES
                         CAMPTOTHECINES ET MEDICAMENTS A EFFETS SECONDAIRES
                         REDUITS
INVENTOR(S):             RATAIN, Mark, J.;
                         GUPTA, Elora
PATENT ASSIGNEE(S):      ARCH DEVELOPMENT CORPORATION
LANGUAGE OF PUBL.:       English
DOCUMENT TYPE:           Patent
PATENT INFORMATION:
                         NUMBER            KIND     DATE
                         ---------------------------------
                         WO 9601127          A1 19960118
DESIGNATED STATES
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                         HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX
                         NO NZ PL PT RO RU SD SE SG SI SK TJ TT UA UZ VN KE MW
                         SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT
                         SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.:       WO 1995-US8394       A  19950705
PRIORITY INFO.:          US 1994-8/271,278       19940705
                         US 1995-8/423,641       19950417


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     (FILE 'HOME' ENTERED AT 11:06:41 ON 28 JUL 2006)

     FILE 'PCTFULL' ENTERED AT 11:06:52 ON 28 JUL 2006
L1          3617 S CAMPTOTHECIN
L2          1569 S L1 AND PRODRUG
L3         89692 S ANTIBOD?
L4          1316 S L3 AND L2
L5        126831 S ENZYME
L6          8323 S ESTERASE
L7           130 S L6 AND L4
L8            74 S ADEPT AND L2
L9             1 S L8 NOT PY>1998
L10         5283 S L6 AND TARGET?
L11          128 S L10 AND L7
L12            3 S L11 NOT PY>1998

=> s l12 and cpt
          2876 CPT
            87 CPTS
          2912 CPT
                 (CPT OR CPTS)
L13          2 L12 AND CPT

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L13    ANSWER 1 OF 2     PCTFULL   COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER:        1998035554 PCTFULL  ED 20020514
TITLE (ENGLISH):         COMBINED TUMOR SUPPRESSOR GENE THERAPY AND CHEMOTHERAPY
                         IN THE TREATMENT OF NEOPLASMS
TITLE (FRENCH):          COMBINAISON THERAPIE GENIQUE SUPPRESSIVE DE TUMEURS -
                         CHIMIOTHERAPIE UTILISEE DANS LE TRAITEMENT DE
                         NEOPLASMES
INVENTOR(S):             NIELSEN, Loretta;
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RESNICK, Gene

LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9835554	A2	19980820

DESIGNATED STATES
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH GM KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1998-US3514 A 19980217
PRIORITY INFO.: US 1997-8/801,285 19970218
US 1997-8/801,681 19970218
US 1997-8/801,755 19970218
US 1997-8/801,765 19970218
US 1997-60/038,065 19970218
US 1997-60/047,834 19970528

L13 ANSWER 2 OF 2 PCTFULL COPYRIGHT 2006 Univentio on STN
ACCESSION NUMBER: 1996001127 PCTFULL ED 20020514
TITLE (ENGLISH): CAMPTOTHECIN DRUG COMBINATIONS AND MEDICAMENTS WITH REDUCED SIDE EFFECTS
TITLE (FRENCH): COMBINAISONS MEDICAMENTEUSE CONTENANT DES CAMPTOTHECINES ET MEDICAMENTS A EFFETS SECONDAIRES REDUITS
INVENTOR(S): RATAIN, Mark, J.;
GUPTA, Elora
PATENT ASSIGNEE(S): ARCH DEVELOPMENT CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9601127	A1	19960118

DESIGNATED STATES
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TT UA UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.: WO 1995-US8394 A 19950705
PRIORITY INFO.: US 1994-8/271,278 19940705
US 1995-8/423,641 19950417

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L13 ANSWER 2 OF 2 PCTFULL COPYRIGHT 2006 Univentio on STN
TIEN CAMPTOTHECIN DRUG COMBINATIONS AND MEDICAMENTS WITH REDUCED SIDE EFFECTS

ABEN This invention provides methods and combination formulations and kits to reduce the toxicity of
camptothecin drugs, such as irinotecan (CPT-11).
Disclosed are therapeutics and treatment methods employing such drugs in combination with agents that increase conjugative enzyme activity or glucuronosyltransferase. . .

DETD DESCRIPTION
CAMPTOTHECIN DRUG COMBINATIONS AND MEDICAMENTS WITH REDUCED SIDE EFFECTS
BACKGROUND OF THE INVENTION
The present application is a continuation-in-part of co-pending U.S. Patent Application Serial. . .
. . .
generally to the fields of reducing drug toxicity and enhancing drug efficacy. More particularly, it concerns new treatment methods, compositions and kits comprising camptothecin drugs, such as irinotecan (CPT-11) and topotecan, in combination with agents that reduce excretion of active
camptothecin species through the bile. Agents that increase conjugative enzyme activity, such as glucuronosyltransferase activity, and agents that decrease poglycoprotein activity, such as cyclosporine A,. . .

Be Description of the Related Art
Camptothecin was identified as the active component of the crude extract from the stem wood of Camptotheca acuminata that showed promising in vitro anti-neoplastic activities by inhibiting topoisomerase 1 (Wall et al., 1966). Camptothecin entered clinical trials in the early 1970s, but these were suspended (Muggia et al., 1972; Gottlieb et al., 1970). The drug exhibited. . . activity and an enhancement of toxicity Mani et al., 1980; Giovanella et al., 1991). The reduced activity was due to the fact that camptothecins require a closed lactone ring structure for optimal activity. Formulation as the sodium salt resulted in opening of the ring to a. . .

Efforts have thus been directed towards synthesis of water-soluble derivatives of camptothecin that would have high antitumor activity and low toxicity. Topotecan was synthesized by the introduction of a basic side chain at the 9-position of the 10-hydroxycamptothecin ring. This enabled topotecan to retain its water solubility in the lactone form (Kingsbury et al., 1991). CPT-11 was synthesized by the introduction of an ethyl group at the 7-position of camptothecin and a hydroxyl group at the lo-position which formed an ester linkage with a piperidinopiperidino carbonyl group Munimoto et al., 1987). The ester. . .

CPT-11 is a water-soluble semi-synthetic derivative that acts as a prodrug in vivo and is converted to SN-38 (7-ethyl-lo-hydroxy-camptothecin) by the enzyme carboxyl
esterase (Tsuji et al., 1991). SN-38 has been shown to undergo glucuronic acid conjugation to form the corresponding glucuronide which is the major. . . deconjugated by the intestinal microflora to form SN-38 (Kaneda et al., 1990). The topoisomerase I inhibition and single strand breaks after treatment with
CPT-11 is determined primarily by SN-38 concentration (Kawato et al,, 1991).

Accumulation of SN-38 in the intestine was shown to be responsible for the diarrhea attributed to CPT-11 administration in nude mice (Araki et al., 1993). Thus the in vivo activity and toxicity of CPT-11 is dependent on SN-38 concentration, and characterization of the disposition of the metabolite following CPT-11 administration is important for designing optimal dosing schedules. Both diarrhea and myelosuppression have been significant concerns, with severe and/or life threatening toxicity being common.

In commenting on the use of CPT-11 and topotecan (TPT), Slichenmyer et al. (1993) proposed that decreased metabolic activation of the CPT-11 pro-drug and active efflux of TPT from the target cells by poglycoprotein-mediated transport might contribute to the resistance to the cytotoxic effects of these agents seen in some cancer .patients. To. . .

(1993) seem to be suggesting that increasing the activation enzyme activity, such as carboxyl esterase, or decreasing target cell poglycoprotein activity may be effective.

However, the Slichenmyer proposals do not offer a solution to the toxicity associated with camptothecins.

Moreover, increasing their metabolic activation, as suggested, may actually increase camptothecin toxicity, absent other methods of intervention. The further proposal of Slichenmyer et al. (1993) to combine camptothecins with other active chemotherapy agents, WO 96/01127 PCTIUS95/08394
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Prior studies have shown inconsistent relationships between the dose or pharmacokinetics of CPT-11 with SN-38 pharmacokinetics and gastrointestinal toxicity ..(Negoro et al., 1991; Ohe et al., 1992; Rothenberg et al., 1993; Rowinsky et al., 1994). Furthermore, there is little information on the metabolic fate of camptothecin drugs in humans, no clear definition of the plasma profile of SN-38G following CPT-11 administration in humans, and no accepted mechanism for reducing camptothecin toxicity.

Despite the ongoing clinical trials, it is clear that a more in-depth understanding of CPT-11 metabolism is needed before safer treatments can be designed.

. . .
these and
other drawbacks inherent in the prior art by providing new treatment methods, compositions and kits for reducing the side effects of camptothecin drugs, such as irinotecan (CPT-11), topotecan and other camptothecin analogues. The invention provides methods for reducing the dose of a camptothecin compound necessary to achieve the same therapeutic benefit, and methods for using more usual dosages, or even increased doses, in order to achieve enhanced therapeutic effects. The methods of the invention generally rest in using a camptothecin compound in combination with an amount of a second agent effective 'to reduce excretion of the active camptothecin species through the bile.

The inventors have discovered that one mechanism

underlying the significant toxicity of CPT-11, for example, is deficient glucuronidation of the CPT-11 metabolite, SN Thè inventors further discovered that the active SN-38 species is transported into the bile, where it causes adverse effects, by the biliary transporter, poglycoprotein. Therefore, advantageous methods for reducing excretion of active camptothecin species, such as SN-38, into the bile include increasing conjugative enzyme activity, such as glucuronosyltransferase activity; and decreasing the activity of biliary, or bile. . . canaliculi, transport proteins, such as poglycoprotein .(FIG. 1), The methods, compositions and kits of the invention may be used in conjunction with any camptothecin drug that has an active species or metabolite that is, at least in part, excreted through the bile. Such camptothecin drugs may themselves be the active species. 9-AC, topotecan and GG211, amongst others, arEi examples of this group of camptothecin analogues.

Alternatively, the camptothecin compound or drug may be one that is metabolized within the body to provide an active species or metabolite, such as CPT-11, which is converted to SN Further examples of this class include 9-nitro-camptothecin, amongst others.

The types and ranges of camptothecin analogues available are well known to those of skill in the art and described in numerous texts. For example, Slichenmyer et al. (1994; 1993), Burris & Fields (1994) and Hawkins (1992), each incorporated herein by reference, review the use of camptothecins. It is contemplated that any of the compounds described in the above texts may be used in -this invention.

Specific examples of active camptothecin analogues include seven-substituted water-soluble camptothecin analogues, as described by Emerson et al. (1995); hexacyclic camptothecin analogues, as described by Sugimori et al. (1994); and 20S configuration camptothecins with substitution at the 9 or 10 positions with amino, halogeno, or hydroxyl groups, 10,11-methylenedioxy substituted camptothecins and water-soluble 20-glycinate ester variants, each described by Wall et al. (1993). Also, E-ring-modified (RS)-camptothecin analogues, such as (RS) deoxyaTino ethyl methoxycamptothecin (Ejima et al., 1992); the water-soluble 9-[(dimethylamino)methyll hydroxycamptothecin of Kingsbury qt al. (1991); and the 9- and 10-substituted camptothecins of Wani et al. (1987, 1986, 1980) may be used.

If desired, camptothecin analogues may be synthesized by following the methodology of, for example, Emerson et al. (1995); Sugimori et al. (1994); Wall et al. (1993);. . .

In certain embodiments, the invention thus provides methods for reducing the toxicity of a camptothecin compound or drug, such as CPT-11, which comprise administering one or more camptothecin drugs in combination with an effective amount of one or more second agents that increase conjugative enzyme activity or that decrease biliary, or. . .

. . .

second agents may simply be
defined as those amounts effective to reduce the side-
effects or toxicity of one or more first camptothecin
drugs when administered to an animal in combination with
the first camptothecin drug(s). This is easily
determined by monitoring the animal or patient and
measuring those physical and biochemical parameters of
health and disease that. . .

In certain embodiments, the doses of camptothecin
drugs, such as CPT-11 and other analogues, used in the
present invention will often be less that those used in
the prior art. Indeed, this is. . . for a smaller dose to be given
in order to achieve the same beneficial anti-cancer or
other therapeutic results. For example, using CPT-11
-with cyclosporine A, allows for about a three-fold
reduction in the CPT-11 dose.

In other embodiments, the doses of camptothecin
drugs administered may be about the same as those
currently used in the art. In such cases, using the
camptothecin compound in combination with a second agent
that reduces biliary excretion of the active species or
metabolite will result in increased bioavailability. . . component.
This may be used, for example, in
patients that have advanced disease or that have proven
resistant to lower doses of camptothecins. Higher
camptothecin levels may also be used, so long a.s.the
second agents are provided in amounts to prevent
significant toxicity or untoward effects in. . .

In any event, as the invention provides for reducing
the toxicity of camptothecin drugs and for increasing the
bioavailability of camptothecin drugs, it will be
apparent that this invention provides for more
variability in the doses of camptothecin species than
previous methods. The attending physician may thus
optimize treatment to the individual patient, effectively
accounting for the variations in disease heterogeneity
that. . .

In further embodiments, the camptothecin drug or
drugs could be administered in combination with both one
or more second agents that increase conjugative enzyme
activity and one or. . . agents) that inhibit biliary transport or
poglycoprotein transport activity. This would give the
added advantage of reducing the biliary excretion of the
active camptothecin species by intervening in two
different metabolic pathways.

It will be understood that the term conjugative
enzyme', as used herein, refers to enzymes that modify
active camptothecin species. These enzymes are distinct
from those activation-type enzymes that metabolize a
pro-drug, such as CPT-11, into an active camptothecin
species, such as SN An example of such as enzyme is
carboxyl esterase.

. . .
glucuronosyltransferase
enzymes, glutathione S-transferase (GST), N-acetyl
transferase, and even quinone reductase (QR) (Prochaska &
Fernandes, 1992). As glucuronidation of SN-38 (an active
metabolite of CPT-11) has been specifically observed in
patients, compounds that increase glucuronosyltransferase
enzyme activity are currently preferred.

. . .
is generally achieved by
inhibiting any membrane transport protein, or protein
complex, that is present in the bile canaliculi..-and that
functions to transport camptothecin analogues. The
inventors discovered that poglycoprotein transports the
camptothecin species CPT-11, SN-38 and SN-38G, and
the
poglycoprotein is thus a preferred target.

In the cancer treatment literature, poglycoprotein
is often referred to in the context of a target cell
protein. Indeed, poglycoprotein contributes to the
multi-drug resistance phenotype observed in cancer cells
by actively pumping drugs out of the cell. This. . . has made the
cellular poglycoprotein the
subject of scientific research and certain studies on
anti-cancer agents. However, it will be understood that
such target cell poglycoprotein studies are distinct
from the approach taken by the present inventors that
concerns biliary transport, i.e., the inventors have
taken a whole animal approach rather than focusing on
events at the ultimate target cells,
Although poglycoprotein has been reported to be
expressed in normal human tissues, such as liver, kidney,
and adrenal gland, its function and. . .
. . .
even reversal of drug resistance in cells in
vitro. Of course, it will be appreciated that animal
testing and pre-clinical studies showing reduced
camptothecin toxicity are the preferred means for
optimizing the invention.
. . .
Barbiturates, such as phenobarbital, which are often
-'used as anti-convulsants, may also be employed as second
agents to activate conjugative enzymes. Indeed, using
CPT-11 and phenobarbital, the inventors found a marked
reduction in toxicity, as shown in Example B.
. . .
or
modify the poglycoprotein transporter (i.e., non-
competitive inhibitors), agents that compete for
poglycoprotein binding sites and render the transporter
less available for the camptothecin drug in question are
is contemplated (i.e., competitive inhibitors).
. . .
et al., 1994) may also be used. Results are
presented herein (Example 19) to show that using
cyclosporine A in combination with CPT-11 is particularly
beneficial. Staurosporine and staurosporine derivatives,
particularly NA-382, may also be employed (Miyamoto
et al., 1992a; 1993).

Antibodies that binds to external epitopes of
poglycoprotein may also be used as second agents to
achieve inhibition. Monoclonal antibodies (MAbs) will
generally be preferred. Many such antibodies are known,
as exemplified by MAb C219 (Miyamoto et al. I 1992b) and
MAbs JSB-1 and C-219 (Miller et al., 1991). The. . . al., 1993). The
MAb MRK16 and the
mouse-human chimeric version MH162 are preferred agents
(Hamada et al., 1990), as is the mouse-human chimeric
antibody, MH171 (Ariyoshi et al., 1992) and the MAb UIC2
(Mechetner & Roninson, 1992). MRK16, MH171 and UIC2 have
been safely used in animals. Other useful monoclonal

antibodies may also be obtained or prepared, so long as the M.Ab generally exhibits binding affinity for external, epitopes of poglycoprotein, as described. . .

The camptothecin class of drugs for use in the invention function to inhibit topoisomerase I and have various therapeutic uses. For example, 9-nitro-camptothecin has been applied in certain situations as an anti-parasitic agent, and camptothecin analogues have well-documented activity against resistant solid tumors, .,particularly colon, lung cancer and ovarian cancer, and refractory leukemia. CPT-11 itself has shown antitumor activity in phase II trials in patients with carcinomas of lung, cervix, ovary, colon, and rectum and in patients with non-Hodgkin's lymphoma. However, it will be appreciated that the camptothecins may be used to treat practically any cancer.

The use of such camptothecin drugs, e.g., CPT-11, has been previously limited by significant gastrointestinal toxicities, including nausea, vomiting and abdominal pain, which side effects can prove fatal.

By modulating camptothecin drug toxicity, the present invention thus also provides improved methods for treating cancers, leukemias, parasitic infections and other diseases and disorders, as desired.

. . .
The treatment methods generally comprise administering to an animal with cancer, including a human patient, a therapeutically effective combination of one or more camptothecin drugs, such as CPT-11, and one or
more second agents that reduce camptothecin toxicity by reducing excretion of the active camptothecin species through the bile, as exemplified by second agents that increase conjugative enzyme activity and/or that inhibit poglycoprotein transport activity. The second agent(s) may. . .

(1990), each incorporated herein by reference, may be employed in connection with the present invention. These studies demonstrated that camptothecin analogues are active against leukemias and metastatic cancers.

In some cases, pre-clinical testing in animals with disease may not be necessary where both the camptothecin drug and the second agent have been previously approved for human treatment. In any event, all that is required to determine or optimize. . .

. . .
Preferably, one would use an amount that resulted in a significant benefit to the patient, as assessed by a significant reduction in camptothecin toxicity or any .increase in the anti-tumor (or anti-parasitic) response.

Animals and patients may also be treated with the camptothecin drug or drugs in combination with two or more second agents, with at least one agent being from the different classes described. . . be described as a second agent and a third
agent. This has the benefit of acting at two distinct points in the camptothecin excretion pathways and may result in further improved or even synergistic effects.

In treatment methods, the first camptothecin drug or drugs, e.g., CPT-11, may be administered to the animal or patient prior to administering the second agent s), or the first drug(s) and the second. . .

It is currently preferred that the second agent be administered to the animal or patient prior to the camptothecin drug(s) in order to prime the system.

Delivery of the second agent prior to the camptothecin drug and continued delivery of the second agent throughout the camptothecin delivery period is one currently preferred treatment method. Delivery in -'these contexts preferably means continuous infusion.

. . .
with agents intended to combat any myelosuppression, such as GCSF (GMCSF), is also contemplated, as is sometimes performed after the administration of a camptothecin drug.

One currently preferred treatment mode comprises administering about 25 mg/m2 of CPT-11 by'infusion over about 90 minutes, about 10 mg/kg cyclosporine A by infusion, and optionally, also about 60 mg/kg of phenobarbital.

. . .
as oral
delivery may be employed, depending on the second agent used to reduce the toxicity or enhance the bioavailability of the first camptothecin drug(s).

. . .
which
are readily determinable without undue experimentation by those of skill in the art. Further publications are available that review the use of camptothecin analogues, -tuch as, e.g., Slichenmyer et al. (1994, 1993) and Hawkins (1992), each incorporated herein by reference.

Also provided are new compositions and formulations, including pharmacologically acceptable formulations, that comprise one or more first camptothecins, such as CPT -11,
in combination with one or more second agents that increase conjugative enzyme activity or that decrease biliary transport protein activity. Such compositions may include the first camptothecin drug or drugs in combination with Oltipraz, clofibrate, ciprofibrate, fenofibrate, bezafibrate, gemfibrazol, tiadenol, probucol, phenobarbital, dilantin, clonazepam, clotrimazole, buthionine sulfoximine (BSO), cyclophosphamide, ifosphamide, a retinoic acid, a corticosteroid, an oral contraceptive, rifampin or disulfiram (Antabuse); and will preferably include CPT-11 in combination with phenobarbital, Oltipraz, all-trans retinoic acid, phenytoin, dexamethasone, rifampin or clofibrate.

The compositions may also include one or more first camptothecin drugs in combination with a cyclosporine or staurosporine, particularly, cyclosporine A or SDZ PSC is 833, NA-382; and/or with verapamil or dex. . .

The compositions may also advantageously include the first camptothecin drug or drugs in combination with one or more second agents selected from the group that

increases conjugative enzyme activity and one. . .
. . .
compositions that
do not produce significant toxicity, detrimental side effects, or other untoward reactions, when given to an .]animal or patient. In that camptothecins such as CPT -11,
prior to the present invention, were known to suffer from certain toxic limitations, it will be understood that pharmaceutically acceptable compositions may still. . .

Therapeutic kits comprising camptothecin drugs, such as CPT-11, and one or more second or third agents'korm another aspect of the invention. Such kits will generally contain, in suitable container means, a pharmaceutical formulation of the camptothecin drug(s), a pharmaceutical formulation of one or more second agents that increase conjugative enzyme activity or that decrease biliary transport protein activity. Multiple agents. . .

is
The invention also provides method for predicting the degree of camptothecin drug, such as CPT-11,
toxicity
that may arise in a patient. One such method generally comprises determining the glucuronidation capacity of the patient, wherein a decreased glucuronidation. . . capacity, in comparison to normal levels, would be indicative of a patient at risk of developing drug toxicity, if a drug such as CPT-11 were to be given alone.
. . .
normal levels, Certain of the diagnostic methods may employ genotyping, i.e., assaying for genetic polymorphisms in enzymes involved in the metabolism of camptothecins, particularly CPT Here, the glucuronidation or biliary transport capacity of the patient is determined by means of determining the amount of DNA or RNA. . .

FIG. 2A and FIG. 2B. Plasma disposition curves for CPT-11 (0), SN-38 (0) and SN-38G W following intravenous infusion of CPT FIG. 2A is a representative plasma profile of a patient having grade 0-2 diarrhea. FIG. 2B is a representative plasma profile of a. . .

FIG. 4A, FIG. 4B, FIG. 4C. Plasma concentrations of CPT-11, SN-38 and SN-38G is control (CPT-11 dose.

20 mg/kg, n=4) versus phenobarbital pretreated rats (n=2), receiving the same dose of CPT Data is represented as mean + SD for the control group vs mean for the pretreated group.

FIG. 5. SDS-PAGE fluorography of [3H]verapamil photcaffinity labeled plasma membranes of MCF-7/Adr cells -'and inhibitory effects of CPT-11 and SN-38 in comparison to verapamil. Cells were labeled with [125,]verapamil in the absence (Lane 1) and presence of 10 AM and 100 AM CPT-11 (Lane 3 and Lane 4), or 10 AM and 100 AM SN-38 (Lane 6 and Lane 7) or 10 AM and 100 AM verapamil (Lane 8 and Lane 9). Both CPT-11 and SN-38 reduce photoaffinity labeling of the cells by competing with [3H]verapamil.

FIG. 6A. FIG. 6B. FIG. 6C. Plasma concentrations of

CPT-11, SN-38 and SN-38G following 20 mg/kg CPT-11 (control, n=4). The pretreated group (n=3) received 60 mg/kg cyclosporine A five minutes prior to the CPT-11 dose. Data is represented as mean + SD.

FIG. 7A, FIG. 7B, FIG. 7C. Plasma concentrations of CPT-11, SN-38 and SN-38G following 10 mg/kg CPT-11 (control, n=3). The pretreated group (n=4) received 60 mg/kg cyclosporine A five minutes prior to the CPT-11 dose. Data is represented as mean + SD.

FIG. 8A, FIG. BB, FIG, 8C. Plasma concentrations of CPT-11, SN-38 and SN-38G following 6 mg/kg CPT-11 (control, n=3). The pretreated group (n=4) received 60 mg/kg cyclosporine A five minutes prior to the CPT-11 is dose. Data is represented as mean + SD.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Camptothecins constitute a group of anti-proliferative agents that possess the ability of inducing single strand breaks in chromosomal DNA and inhibiting nucleic acid synthesis. At concentrations of about 0.5 AM, camptothecin produces instantaneous and reversible nicks in the DNA in presence of the enzyme DNA topoisomerase 1, a novel target for cancer chemotherapy (Hsiang et al., 1985).

. is followed by a break in the DNA strand thereby promoting passage of the unbroken strand. The break is then resealed by topoisomerase. Camptothecins block this resealing step by forming a ternary complex with the DNA and topoisomerase I resulting in an accumulation of cleavable complexes and. . .

The cytotoxicity of camptothecins has been shown to be optimal at the S-phase compared to the G -G 1 2 phase suggesting that the single stranded breaks. . . Li et al., 1972). The level of topoisomerase 1 activity has been reported to be elevated in tumor specimens making them selective targets for camptothecin activity (Giovanella et al., 1989).

CANEMTHECIN ANALOGUES

Rj]]
OH
N
A
R3 R2 0
OH
N 0
B
0
0
OH
N 0
c
HO
Ir CH3 0
CH3 9HCI
D CIN-C
HCI M20
0
CH3,
N

E
N 0
NH2 0

Camptothecin analogues are generally based upon the structure shown in A, to which various R groups may be added. Structure B is camptothecin itself; C is topotecan; D is CPT-11; and E is 9-amino-camptothecin (9-
AC). The camptothecins of structures B through E are only exemplary forms of camptothecins that may be used in the present invention. Numerous other camptothecin analogues are available, as described by, e.g., Slichenmyer et al. (1994; 1993), Burris & Fields (1994) and Hawkins (1992).

Specific examples of active camptothecin analogues are 9-nitro-camptothecin (with a nitro group at position 9, rather than the amino group shown in structure E); GG211; seven-substituted water-soluble camptothecins (Emerson et al., 1995); hexacyclic camptothecin analogues (Sugimori et al., 1994); nine or ten-substituted camptothecins (Wani et al., 1987;1986;1980; Wall et al., 1993; Kingsbury et al,, 1991); and E-ring-modified camptothecins (Ejima et al., 1992).

(1976) each describe effective methodology for synthesizing camptothecin analogues. However, many are commercially available from different sources. For example, topotecan is generally supplied as the hydrochloride salt in a lyophilized mixture. . .

9-amino-20(s)-camptothecin (9-AC) is another camptothecinanalog that has demonstrated potent preclinical anti-cancer a ctivity. Human xenografts studies have revealed significant activity of 9-AC against colon cancer.. . .

Irinotecan (CPT-11) exhibited significant activity in a broad spectrum of in vitro and in vivo tumor models.

CPT-11 is converted in vivo to its active metabolite, SN-38, which is 600-1000 times more potent than the parent drug. Both CPT-11 and SN-38 occur as open and closed lactone forms after administration.
. . .
the closed lactone ring forms possess significant antitumor activity. The terminal half-life for the closed ring forms ranged from 5 6.1 hours for CPT-11 and 7 8.8 hours for SN In animal studies, CPT-11 was found to be equally excreted into bile and urine while SN-38 was excreted primarily into bile.

- 34
Several Phase I studies of CPT-11 have already been conducted in Japan and Europe and at two centers in the United States. In these studies, CPT-11 has been administered as a 90 minute IV infusion on a variety of schedules including weekly x 4, alternating weeks x 2, every 4 weeks, daily x 5 q 4 weeks,*BID x 7 days q 4 weeks and daily x 3 q week. CPT-11 has also been administered as a continuous infusion for 5 days every 4 weeks. In the Phase I studies, the DLT was. . . diarrhea. other gastrointestinal toxicities such as nausea, vomiting and abdominal pain have also been observed. There have been inconsistent results regarding pharmacodynamic

correlations between CPT-11 and/or SN-38 AUC and diarrhea and/or myelosuppression.

Based upon this information, the inventors initiated a Phase I study of CPT-11 administered as a 90 minute infusion weekly x 4 in a six week cycle. They hypothesized that with G-CSF and optimal pharmacologic management. . .

A total of 25 patients have been treated since study activation (July 29, 1993) at CPT-11 doses ranging from 100-175 mg/m2. The detailed examples presented in the present application reflect treatment on 21 patients.

. . .

dose reduction for
cycle 2. Patient 15 (at 145 mg/m2) also had grade 3 abdominal cramping and declined a second cycle of CPT Four other patients had mild (grade 1) abdominal cramps.

. . .

weeks 4 and
3, respectively of cycle 1. Beginning with patient 17, all patients received G-CSF for 5 days following each dose of CPT No cases of neutropenia > grade 1 were observed at 145 mg/m2 following this intervention.

Four separate HPLC assays are being utilized in this study. They measure open and closed CPT-11, open and closed SN-38, total CPT-11 and SN-38, and SN-38 glucuronide. In addition to plasma concentrations, the inventors are collecting urine for the first 24 hours after the first. . .

Fresh plasma transported on ice is immediately analyzed for open vs. closed (lactone ring) CPT-11 and SN-38 by a modification of the method of Rothenberg et al. (1993). Samples are prepared from 250 AL of plasma. The proteins are precipitated by adding 500 AL of cold methanol (-200C) followed by centrifugation. For open versus closed CPT-11 analysis, a dilution of 150 AL of the supernatant is made with 150 AL of 0.1 M sodium dihydrogen phosphate with 3. . .

. . .

drug is
relatively constant, there is relatively little advantage to measuring both active and inactive species, as opposed to total drug. Since for CPT-11 and SN-38, such assays are quite labor-intensive, the inventors have amended their study to only perform such detailed assays on a small subset. . .

The inventors assayed for total CPT-11 and SN-38 using standard intensive sampling, applying a modification of the assay of Chabot et al, (1992). A liquid-liquid extraction is used, as. . .

CPT-11 and SN-38 are extracted using cold methanol (2 mL) and evaporated to dryness under nitrogen. The residue is reconstituted with 250 AL. . .

. . .

would be an important parameter. To control
for interindividual variability in the amount of available drug, this ratio was multiplied by the CPT-11 AUC to obtain this biliary index.

. . .

0-2 diarrhea (n=12) and Grade

3-4 (n=9) diarrhea. The most important pharmacokinetic parameter appears to be the biliary index ($P<0.0004$), derived from the CPT-11, SN-38, and SN-38 glucuronide AUCs. Using only the ten patients treated at a uniform dose of 145 mg/m2, the biliary index was. . .

Since glucuronidation appears to be polymorphic and potentially typeable, the present inventors propose that the development of CPT-11 may be similar to that used for amonafide. The first step will be to extend the present .studies showing that patients that are poor glucuronidators are at higher risk of CPT-11 induced diarrhea. In conjunction with these studies, the inventors will correlate SN-38 glucuronidation with a glucuronidation probe. Acetaminophen, which undergoes an ether glucuronidation, as. . . blacks may have a higher incidence of poor
glucuronidators (Hecht et al., 1994), it will be especially important to evaluate interracial differences in CPT-11 toxicity. Again, the inventors have previously demonstrated that minority patients are at greater risk for amonafide toxicity. Because of the potential intergender and. . .

The inventors propose that a correlation between glucuronidator status and CPT-11 toxicity exists and that it is thus very important to induce this phase II enzyme.
. . .
in a rodent model (Egner
et al., 1994). Therefore oltipraz is considered as a potential agent with which to induce glucuronidation prior to CPT-11 treatment, and monitored with acetaminophen phenotyping. Such a strategy is contemplated to significantly enhance the therapeutic index of CPT
Since gastrointestinal toxicity was related to excessive amounts of SN-38 in the bile that drained into the gut, the inventors realized that another approach to reduce toxicity of camptothecins, such as CPT-11, would
be to reduce transport into the bile (FIG. 1). The inventors thus planned a study to identify the biliary component responsible for the camptothecin transport activity. it was reasoned that the poglycoprotein transporter was one candidate. The poglycoprotein is located in the canalicular membrane of hepatocytes and is believed to be involved in the biliary transport of several compounds. The inventors determined that both CPT-11 and SN-38 did interact with the poglycoprotein.

The inventors thus reasoned that poglycoprotein inhibitors would be useful agents for administration in combination with camptothecins. Using animal studies, it was found that both high and low doses of cyclosporine A (cyc A) increased the systemic availability of CPT-11, indicating a reduction or inhibition of biliary excretion. Therefore pretreatment with poglycoprotein inhibitors, such as cyclosporine A, is proposed to be a useful means of decreasing toxicity and plasma clearance of CPT-11 and improving the bioavailability of SN
The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated. . .
. . .
the

standard laboratory criteria including criteria for adequate organ function. Informed, written consent was obtained from all patients prior to their first dose of CPT The drug was given in 500 cc normal saline by intravenous infusion over 90 minutes on a weekly basis for four doses. . .

Stool collections were also obtained to test for any co-existing infection. CPT-11 doses were held until diarrhea resolved < grade 2.

. . .

metabolite levels, heparinized
blood samples obtained on the first cycle of therapy, were centrifuged and the plasma was stored at -700C until analysis. CPT (camptothecin, 1 gg/ml, obtained from the
National Cancer Institute, Bethesda, MD) was used as an internal standard. one hundred Al of plasma was....extracted with. . .

The total CPT-11 and SN-38 concentrations in the plasma were estimated by modification of the HPLC method of Barilero et al. (Barilero et al., 1992).. . .

. . .

Hitachi F1050 Fluorescence
detector (Hitachi Instruments Inc., Naperville, IL) with a Xex at 375 nm and Xem at 566 nm. Standard curves of CPT-11 (obtained from Yakult Honsha Co. Ltd., Tokyo, Japan) and SN-38 (obtained from Yakult Honsha Co. Ltd.) were linear within the range of. . .

EXAMPLE 4
Data Analysis
The plasma concentration-time data of CPT-11, SN-38 and SN-38G were analyzed by non-compartmental analysis using PCNONLIN (SCI, Lexington, KY). The area under the plasma concentration-time curve (AUC) from time. . .

Since CPT-11 induced diarrhea in nude mice was associated with intestinal accumulation of SN-38 (Araki et al,, 1993), biliary concentrations of the metabolite might be. . . SN-38 to SN-38G. To control for individual variability in the amount of available drug, the ratio was multiplied by the AUC of CPT-11 to obtain a biliary index of SN This was expressed as.

. . .

in the bile
draining into the gut, and would be at a higher risk of gastrointestinal toxicity. Also, patients receiving high doses of CPT-11, may have saturation of the glucuronidation pathway, leading to elevated biliary SN-38 concentrations. Overall, the higher the biliary index of a patient,. . .

. . .

differences in pharmacokinetic outcomes between two patient groups, defined by the worst severity of diarrhea experienced in the first two cycles of CPT-11 treatment. Statistical tests were performed in the Number Cruncher Statistical System (Dr. Jerry Hintz, Kaysville, UT). A two-sided significance level of less than or. . .

EXAMPLE 5
Metabolism of CPT-11
Following intravenous infusion of CPT-11, two

metabolites could be detected in the plasma: SN-38 and the SN-38G. The glucuronide was the major metabolite, with peak plasma concentrations occurring. . .
. .
in agreement with preclinical studies in rats that reported 550i, 220]c and 9% of the biliary radioactivity excreted over 24 h was .,unchanged CPT-11, SN-38G and SN-38 and approximately 18% of the biliary radioactivity was reabsorbed from the intestine (Atsumi et al,, 1991). Pharmacokinetic estimations of the. . . in the four dose levels are listed in Table 1. There was no effect of pretreatment with G-CSF on the pharmacokinetics of CPT-11 and its metabolites. A nonlinear 2.6 fold increase of AUC of CPT-11 from the 100 mg/m2 to the 175 mg/m2 dose level correlated to the decrease in CL estimates and was in accordance with previous reports of nonlinear pharmacokinetics of CPT-11 (Kaneda et al., 1990; Negoro et al., 1991; Kaneda & Yokokura, 1990). However, there was also a 3.7 and 2.7 fold increase. . . appeared to be no increase in the SN-38G AUC between the 145 mg/m2 and the 175 mg/m2 dose levels. The nonlinear increase in CPT-11 AUC seen in the present study could be due to progressive saturation of both the non-metabolic and metabolic pathways of elimination of CPT The plateau concentrations of SN-38G at the two highest dose levels indicate saturation of glucuronidation of SN-38 to SN-38G. The increase in the SN-38 AUC irrespective of decreasing CL of CPT-11 could be due to the capacity limitation of the glucuronidation pathway of SN The secondary peak in the plasma profile contributing to about. . . increase in the AUCSN-38 is suggestive of hydrolysis of SN-38G by glucuronidase resulting in enterohepatic circulation of SN

TABLE le
Pharmacokinetic estimates of CPT-11, SN-38 and SN-389 by dose level. Data are represented as mean ± SD.

Dose Level	AUC CPT-1 1	AUC SN-38	AUC SN-389 2	CL
	ng.himl	ng.himl	ng.himl	literthim
1 00 Mg/M2	5603±967	102.4±28	399.4±344	20.31 ±4.37
n=3				
120 mg/m2	5031±1111	127.4±45	266.9±233	24.93±5.98
n=6				
145 mg/m2	11972±6790	271.2±119	1152±1199	13.91±5.98
n-10				
175 Mglm2	14543±5220	376.1±6.29	1058±622	12.86±4.62
L n=2				

-

TABLE 2*
Correlation of pharmacokinetic estimate to CPT-induced Parrhea. Patients receiving a dose of 145 mg/m were classified according to ihe Pharmacokinetic Grade 0-2 Grade 3-4 p-Value
Estimate n=5 n=5
AUCCPT-1. . . 2i to 44% variability in the AUCSN-38 estimates as measured by the percent .,coefficient of variation. It has been suggested that variability in CPT-11 disposition was due to interpatient

differences in carboxyl esterase levels (Negoro et al., 1991; Ohe et al., 1992; Rothenberg et al., 1993; Rowinsky et al., 1994). However, estimation of carboxyl esterase activity in predose plasma samples of patients in this study showed poor correlation to dose normalized AUC of SN-38 or summation of SN-38 and SN-38G (Gupta et al., 1994a). This indicated that formation from CPT-11 was not the rate determining step in the disposition of SN-38. Moreover, on average 0 1v and 30i of the dose. . . . . c diff +) which likely contributed to the severity of diarrhea. Hence,, in agreement with the inventors, hypothesis, with respect to the total CPT-11 available to the systemic circulation, patients with relatively low glucuronidation rates had progressive accumulation of SN-38 leading to toxicity. The hypothesis was supported. . . . . treatment-related toxicities of several anti-cancer drugs (Lennard et al., 1989; Harris et al,, 1991; Ratain et al., 1991). In the case of CPT-11, variability in glucuronidation, which may be genetic, was primarily responsible for differential accumulation of SN-38 in the gut. Since glucuronidation represents the major detoxification. . . the toxicity of drugs such as acetaminophen (De Mordis & .,Wells, 1989; Hjelle, 1986). Therefore, one approach to increasing the therapeutic index of CPT-11 would be to induce glucuronosyl transferase activity.

EXAMPLE 8

CPT-11 AND PHENOBARBITAL ADMINISTRATION

The isoforms of UDP-GT can be broadly classified based on differential induction by phenobarbital and 3-methylcholanthrene (Burchell & Coughtrie, 1989).. . .

A* Materials and Methods

Materials: CPT-11 solution was obtained from the Yakult Honsha Co., Tokyo, Japan, phenobarbital was obtained from Elkins-Sinn Inc., Cherry Hill, NJ. All other chemicals were. . .

(w/V) phenobarbital in drinking water for four days. on day 5, CPT-11 (20 mg/kg) was administered through the .'jugular catheter and blood samples were withdrawn and analyzed as described later. The control animals received a single dose of CPT-11 (20 mg/kg) only. . . . . blood samples (200 Al) were withdrawn through the catheter at 3, 5, 10, 15, 30, 60, 120f 240 and 260 minutes following CPT-11 administration. After each sample the catheter was flushed with an equal volume of physiologic saline. The samples were immediately centrifuged at 2500 X g for 10 minutes and the plasma so obtained was stored at -700C until analysis. CPT-11, SN-38 and SN-38G in 10-20 Al plasma samples were quantitated by a the reversed-phase HPLC method as previous.1y described (Gupta et al.,. . . . . concentration-time profile of the control and pretreated groups of rats were analyzed using non-compartmental methods (PCNONLIN, SCI, Lexington, KY). The systemic exposure of CPT-11 and metabolites was estimated as the area under the

concentration-time curve from time 0 to 6 hours (AUC).

. . .

induced animals
compared to the controls (Table 3, FIG. 4A, FIG. 4E, FIG. 4C). On the other hand, the AUC of both CPT-11 and SN-38 indicated a 60% and 50% decline. The decline in CPT-11 concentrations was possibly due to enhanced deesterification of CPT-11 to SN The resultant pool of SN-38 however, was insufficient to compensate for the increased substrate requirement by the induced UDP-GT which therefore resulted in a 50% decline in the AUC of SN The overall decline in the AUC of both CPT-11 and SN-38 was indicative of increased conjugation of SN-38 by UDP-GT.

-

TABLE 3
EFFECT OF INDUCTION OF UDP-GT ACTIVITY
GROUP AUCCPT-1 1 AUCSN-38 AUCSN-38G
ng.hriml ng.hriml ng.hriml
CPT-1 I : 20 mglkg
Phenobarbital: 75
mglkgld f or 3 days
(FIG, 4A. B & C)

---Logging off of STN---

Control (nm4) 7695.8±2674 364.B9±44.19
556.52±167.03
Pretreated (n = 2) 3055.8 182.07 1521.7
Change 29% 1% 173
Effect of pretreatment with phenobarbital on the disposition of CPT Control rats were given a single bolus dose of 20 mg/kg CPT Pretreated rats received a single intraperitoneal administration of phenobarbital (100 mg/kg) and 0.1]; (w/v) phenobarbital in drinking water for 4 consecutive days. on day 5 the pretreated rats were given a single bolus dose of 20 mg/kg CPT Data is represented as mean + SD for the control group and mean for the pretreated group.

. . .

by phenobarbital resulted in a 2.7 fold increase in the SN-38G concentration in conjunction with about a 2 fold decrease in the CPT-11 and SN-38 availability. This would result in a substantially lower concentration of SN-38 draining into the gut.

. . .

starting'points
for doses of second agents that increase conjugative enzyme activity. The doses described may be considered for therapeutic use in combination with CPT-11, however, such doses are not intended to be optimum values, but to provide a range that can be readily optimized by a physician. . .

. . .

Schnell, 1991;
Egner et al., 1994). Therefore oltipraz is considered as a potential agent with which to induce glucuronidation prior to, or with, CPT-11 treatment. Doses such as those described by Ansher et al., 1983; Davies & Schnell, 1991; and Egner et al., 1994; each incorporated. . .

TABLE 5

Steroidsi for use in targeted angiogenesis inhibitors
Tetrahydrocorticosterone Tetrahydrocortexolone
Cortisol (hydrocortisone) Prednisone
Tetrahydrocortisol Triamcinolone
11 a-epihydrocortisol Alclometasone
is Cortisone Amcinonide
Tetrahydrocortisone Clobetasol
Corticosterone Clobetasone
Deoxycorticosterone Clocortolone
Cortexolone Desonide
Beclomethasone Desoximetasone
dipropionate
Betamethasone Diflorasone
Dexamethasone Fluocinolone acetonide
Flunisolide Fluocinonide
Methylprednisolone Fluorometholone
17. . .

EXAMPLE 18
POGLYCOPROTEIN BINDING
To determine whether CPT-11 and SN-38 interact with poglycoprotein, membrane vesicles from the multidrug resistant breast tumor cell line MCF-7/Adr were used and the inhibitory effects on. . .
. . .
photolabeled with 50 nM of a photoaffinity
125
analog of verapamil, N-(p-azido-[3,5- Ilsalicyl)-verapamil ([125I]BAS-VPI in the absence or presence of increasing concentrations of CPT-11, SN-38 or nonlabeled verapamil (positive control. Immunoprecipitation of [12-5,]verapamil was performed with a monoclonal antibody specific for P-gp. Proteins were separated by 5-1506 sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and detected by fluorography.

CPT-11 (10 AM) and SN-38 (100 AM) inhibited poglycoprotein affinity labeling by 80!k and 60-0,5, respectively, suggesting that both compounds were substrates for poglycoprotein. . .

EXAMPLE 19
CPT-11 AND CYCLOSPORINE A ADMINISTRATION
Poglycoprotein is a 170-180 kDa membrane glycoprotein that functions as an ATP-dependent transmembrane efflux pump and is considered to. . . et al,, 1992; Okamura et al,, 1993;
Zacherl et al,, 1994; Charuk et al., 1994). The studies in Example 18 demonstrated that CPT-11, SN-38 as well as SN-38G were substrates of poglycoprotein. The objective of the present study was to determine the effects of poglycoprotein inhibition by cyclosporine A on the biliary transport of CPT-11 and its metabolites.

Ao Materials and Methods
Materials: CPT-11 solution was obtained from the Yakult Honsha Co., Tokyo, Japan, and cyclosporine A (Sandimmune) was purchased from Sandoz Pharmaceutical Co., East Hanover, NJ.. . .

To investigate the effect of cyclosporine A on CPT-11 disposition, the animals were divided into two groups.

The control group received an intravenous bolus administration of CPT-11 through the catheter at doses of

6 mg/kg, 10 mg/kg or 20 mg/kg. The pretreated group of rats received an intravenous bolus injection of cyclosporine A (60 mg/kg) five minutes prior to receiving the dose of CPT The catheter was flushed with 500 pl of physiologic saline after each drug administration.

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Serial blood samples (200 Al) were withdrawn through the catheter at 3, 5, 10, 15, 30, 60, 120, 240 and'260 minutes following CPT-11 administration. After each sample the catheter was flushed with an equal volume of physiologic saline. The samples were immediately centrifuged at 2500 X g for 10 minutes and the plasma obtained was stored at -700C until analysis. CPT-11, SN-38 and SN-38G in 10-20 Al plasma samples were quantitated by a the reversed-phase HPLC method as previously described (Gupta et al.,,.

concentration-time profile of the control and pretreated groups of rats were analyzed using non-compartmental methods (PCNONLIN, SCI, Lexington, KY). The systemic exposure of CPT-11 and metabolites was estimated as the area under the concentration-time curve from time 0 to 6 hours (AUC).

B* Results

Cyclosporine A significantly elevated plasma concentrations of CPT-11, SN-38 and SN-38G compared to control plasma levels at all three dose levels of 20 mg/kg, 10 mg/kg and 6 mg/kg (FIG..

onset of the progressive increase in the concentrations occurred between 15 to 30 minutes following CPT-11 dose suggestive of a time lag between cyclosporine dose and poglycoprotein inhibition. The elevations appeared to be independent of the CPT-11 dose with a net increase in AUC across the 3 dose levels being about 3 fold for both CPT-11 and SN-38 and about 2 fold for SN-38G (Table 6; Table 7).

-

TABLE 6

EFFECT OF INHIBITION OF BILIARY EXCRETION ON CPT-11 DISPOSITION

GROUP	AUC CPT-1 I ng.hriml	AUC SN-38 ng.hrimi	AUC SN-38G ng.hriml
CPT-11 1: 20rngjkg	7695.8±2674	364.89±44.19	556.52±167.03
Cyclosporine: 60 mglkg	19722±2631	1052.0±32.29	1062.4±83.54
IFIG, 6A, B & M	2.56 fold	2.88 fold	1.91 fold
Control (n=4)			
Pretreated (nm3)			
Increase			
CPT-11: 10 mglkg	3130.3±726.8	200.66±65.85	354.08±44.57
Cyclosporine: 60 mglkg	3 617.29±100.1	729.16±365.45	(FIGo 7A. B & C) 10808±3229 5
2.06 fold	3.45 fold	3.08 fold	
Control (n=3)			
0 Pretreated (n=4)			
Increase			
CPT-11: 6 mglkg	1728.6±99.32	198.0±21.96	241.92±87.55

Cyclosporine: 60 rngjkq 5392.9±2109 497.33±52.09
440.68±209.1
(FIG, OA, B & M 3.12 fold 2.51 fold 1.82 fold
Control (n=3)
Pretreated (n=4)
Increase
Overall Increase 3.04 fold 2.82 fold 1.93 fold
Effect of cyclosporine A on the disposition of CPT-
11 and metabolites in rats. The control group received
an intravenous bolus CPT-11 dose of 20, 10 or 6 mg/kg.

The pretreated group received an intravenous bolus of
cyclosporine (60 mg/kg) five minutes before receiving
CPT The increase in the bioavailability of CPT-11,
SN-38 and SN-38G was calculated as the ratio of the 6 hr
AUC of the pretreated group to the control group. The
overall. . .

-
TABLE 7
EFFECT OF INHIBITION OF BILIARY EXCRETION
ON CPT-11 DISPOSITION
Effect of lower dose of cyclosporine
GROUP AUCCOT-1 i AUCSN-38 AUCSN-38G
ng.hriml ng.hriml ng.hriml
CPT-11: 10 mglkg 3130.3i726.83 200.66±65.85
354.08±44.57
Cyclosporine: 30 mglkg 12147 413,58 844.43
3 fold 2.06 fold 2.38 fold
Control (n=4)
Pretreated (n=2)
Increase
The increase in the bioavailability of CPT-11, SN-38
and SN-38G (reflected by the increased in AUC) following
cyclosporine administration was secondary to reduction of
inhibition of poglycoprotein associated transport.

. . .
tubular cells in the
kidney, reduction in both biliary and renal excretion
could shave contributed to the decrease in total body
clearance of CPT-11 and metabolites, resulting in
significantly increased AUC. However, biliary excretion
is the major pathway of elimination of CPT-11, SN-38 and
SN-38G with only about 20% of a CPT-11 dose being
eliminated in the urine in rats (Kaneda & Yokokura,
1990). Therefore, reduction in biliary excretion
probably had the major contribution to the reduced
clearance and is reflective of the significant
contribution of this excretion pathway to the overall
total body clearance of CPT-11 and metabolites.

. . .
humans, the plasma half-life
of cyclosporine has been reported to be about 4 to 6
hours. Hence the effect of poglycoprotein inhibition of
CPT-11 disposition could be expected to be observed for a
maximum period of 24 hours following cyclosporine
delivery, during which time about 90k. . .

. . .
concentration and increase
in plasma concentration of SN-38, would not only result
in reduced toxicity but would also enhance the
therapeutic efficacy of CPT Higher plasma SN-38 WO 96/01127
PCrIUS95/08394
72
for reducing toxicity and enhancing the therapeutic index

of CPT Even more attractive is the combined use of cyclosporine, phenobarbital and CPT
Cyclosporine A is currently proposed for use at 10 mg/kg by a 6-hour infusion, and CPT-11 at 25 mg/m2 by a 90-minute infusion beginning about 3 hours after the initiation of cyclosporine infusion.

. . .

that decrease biliary
transport protein activity, as exemplified by decreasing poglycoprotein activity. The doses described may be considered for therapeutic use in combination with camptothecins such as CPT-11, however, such doses are not
intended to be optimum values, but to provide a range that can be readily optimized by a. . .

Boesch et al. (1991) also showed that SDZ PSC 833 was an effective inhibitor. In studies with target cells, PSC 833 was at least one order of magnitude more active than cyclosporine A in restoring drug sensitivity of multi-drug resistance.(MDR) P388. . .

. . .

due to inhibiting drug binding to
poglycoprotein. Therefore, both staurosporine and NA-382 may also be used as second agents in accordance with camptothecins.

. . .

is also similar to Nicardipine, with
properties close to nifedipine, and having effective antianginal and antihypertensive uses. Nisoldipine is proposed for use with camptothecins in the present invention, in a similar manner to nicardipine and nifedipine.

. . .

of tumor resistance in vivo Hait
et al., 1993). The present inventors thus propose that trans-flupenthixol would be particularly effective for combination with camptothecins, as disclosed herein.

Binding and inhibition of poglycoprotein may thus occur at distinct sites on the molecule. This is also supported by monoclonal antibody data.

In light of the studies of Ford et al. (1990) and Akiyama et al. (1988), prochlorperazine is envisioned for use with camptothecins in the present invention.

The antibody studies of Mechetner & Roninson (1992) and.

. . .

concentrations that may
inhibit poglycoprotein function can be achieved is relevant to the present invention, allowing tamoxifen to be used in conjunction with camptothecins.

. . .

study, and
has a relatively low toxicity profile, this drug and drugs of its class are currently preferred for use in combination with camptothecins in the present invention.

EXAMPLE 29
MONOCLONAL ANTIBODIES
Ae MRK16 and MH162
A first mouse-human chimeric antibody against poglycoprotein was developed by Hamada et al. (1990) in an effort to devise an effective treatment for human

drug-resistant cancers. The recombinant chimeric antibody has the antigen-recognizing variable regions of MRK16 joined with the constant regions of human antibodies. When human effector cells were used, the chimeric antibody, MH162, was more effective in killing drug-resistant tumor cells than the all-mouse monoclonal MRK16. As MRK16 inhibited the growth of human drug-resistant. . .

Be MR171
A second mouse-human chimeric antibody, MH171, against poglycoprotein was developed by Ariyoshi et al.

(1992), in which antigen-recognizing variable regions of the mouse monoclonal antibody MRK17 are joined with the constant regions of human IgGi antibodies. MRK17 specifically recognizes poglycoprotein and inhibits the growth of human multidrug resistant (MDR) tumor cells in vitro and in the xenograft nude mouse. . .

MRK17, MH171, MRK16 and MH162 are envisioned for use as an effective second agent for treatment with camptothecins, particularly as success has been demonstrated for other applications in animal models.

De Further MAbs
Schinkel et al. (1993) described the binding properties of MAbs that recognize external epitopes of human poglycoprotein. Such antibodies may he used as specific inhibitors of poglycoprotein-mediated multidrug resistance. Schinkel et al. (1993) particularly describe the MAbs MRK16, HYB-241, UIC2, 7G4 and 4E3, and one of which may be used to inhibit poglycoprotein in combination with camptothecins.

EXAMPLE 30
PHARMACEUTICAL COMPOSITIONS
Aqueous compositions of the present invention will have an effective amount of CPT-11 and an effective amount of a compound (second agent) that increases conjugative enzyme activity, as represented by a compound .that increases the activity. . .

. . .
for
parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. The preparation of an ..aqueous composition that contains CPT-11 and a second agent as active ingredients will be known to those of skill in the art in light of the present. . .

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CLMEN 1 The use of an agent having the ability to reduce excretion of an active camptothecin species through the bile in the preparation of a medicament for use in

reducing the toxicity of a camptothecin compound upon administration of the compound to an animal.

2 The use of an agent in the preparation of a medicament for use in reducing the toxicity of a camptothecin compound upon administration to an animal, the agent present in the medicament in an amount is effective to reduce excretion of an active camptothecin species through the bile upon administration of the medicament and the camptothecin compound to the animal.

3 A use according to claim 1, wherein said camptothecin compound is a camptothecin analogue having a substitution at the 7, 9 or 10 positions.

4 A use according to claim 1, wherein said camptothecin compound is topotecan, 9-amino-camptothecin (9-AC), 9-nitro-camptothecin, GG211 or CPT-11 (irinotecan).

5 A use according to claim 4, wherein said camptothecin compound is CPT

6 The use of an agent having the ability to:
increase the activity of a conjugative enzyme; or
decrease the activity of a biliary transport protein;
in the preparation of a medicament for use in reducing the toxicity of a camptothecin compound upon administration of said camptothecin compound to an animal.

32 A use according to claim 19, wherein said agent is an antibody that binds to poglycoprotein.

34 The use of an agent having the ability to reduce the biliary excretion of an active camptothecin species in the preparation of a medicament for use in treating cancer or a parasitic infection upon combined administration of the medicament and the camptothecin compound to an animal with cancer or a parasitic infection.

. .
or a parasitic infection, the agent present in the medicament in a therapeutic amount effective to reduce the biliary excretion of an active camptothecin species upon combined administration of the medicament and a camptothecin drug to an animal with cancer or a parasitic infection.

36 A use according to claim 34, wherein said camptothecin drug is topotecan, 9-amino-camptothecin (9-AC), 9-nitro-camptothecin, GG211 or CPT

37 A use according to claim 34, wherein said medicament is intended for administration to the animal prior to said camptothecin drug.
. A use according to claim 34, wherein said medicament or said camptothecin drug are administered parenterally.

39 A use according to claim 34, wherein said medicament or said camptothecin drug are administered orally.

the preparation of a medicament for use in treating cancer or a parasitic infection upon combined administration of the medicament and a camptothecin compound to an animal with cancer or a parasitic infection.

49 A use according to claim 48, comprising
.2
administering about 25 mg/m of CPT-11 by infusion over about 90 minutes, about 60 mg/kg of phenobarbital and about 10 mg/kg cyclosporine A by infusion.

50 A composition comprising a camptothecin drug in combination with an agent that increases conjugative enzyme activity or that decreases biliary transport protein activity.

51 The composition of claim 50, wherein said camptothecin drug is topotecan, 9-amino-camptothecin (9-AC), 9-nitro-camptothecin, GG211 or CPT

58 The composition of claim 50, comprising CPT-11 in combination with a first agent selected from the group consisting of phenobarbital, Oltipraz, all-trans retinoic acid, phenytoin, dexamethasone, rifampin and clofibrate, and a. . .

60 A therapeutic kit comprising, in suitable container means, a pharmaceutical formulation of a camptothecin drug and a pharmaceutical formulation of an agent that increases glucuronosyltransferase enzyme activity or-that decreases poglycoprotein transport activity.

61 The kit of claim 60, wherein said camptothecin drug and said agent are present within a single container means,

62 The kit of claim 60, wherein said camptothecin drug and said agent are present within distinct container means.

64 The kit of claim 60, wherein said camptothecin drug is CPT

67 The kit of claim 60, comprising CPT-11, a pharmaceutical formulation of a first agent selected from the group consisting of phenobarbital, Oltipraz, all-trans retinoic acid, phenytoin, dexamethasone, is rifampin and clofibrate,. . .

68 A method for predicting the degree of camptothecin drug toxicity in a patient, comprising obtaining a biological sample from the patient and testing the sample to determine the glucuronidation or biliary. . . a decreased glucuronidation capacity or an increased biliary ..transport capacity, in comparison to normal levels, is indicative of a patient at risk of camptothecin drug toxicity.

69 The method of claim 68, further defined as a method for predicting the degree of CPT-11 toxicity in a patient.

71 The use of a glucuronidatable substrate in the preparation of a formulation for use in predicting the

degree of camptothecin drug toxicity in a patient.

=>

Executing the logoff script...

=> LOG Y

ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:
LOGOFF? (Y)/N/HOLD:
'LOG Y' IS NOT VALID HERE
For an explanation, enter "HELP LOGOFF".

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	33.12	33.33

STN INTERNATIONAL LOGOFF AT 11:17:32 ON 28 JUL 2006

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1642BJF

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

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NEWS	7	MAY 30	IPC 8 Rolled-up Core codes added to CA/CAplus and USPATFULL/USPAT2
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